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A Journal of Pulmonary Diseases

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MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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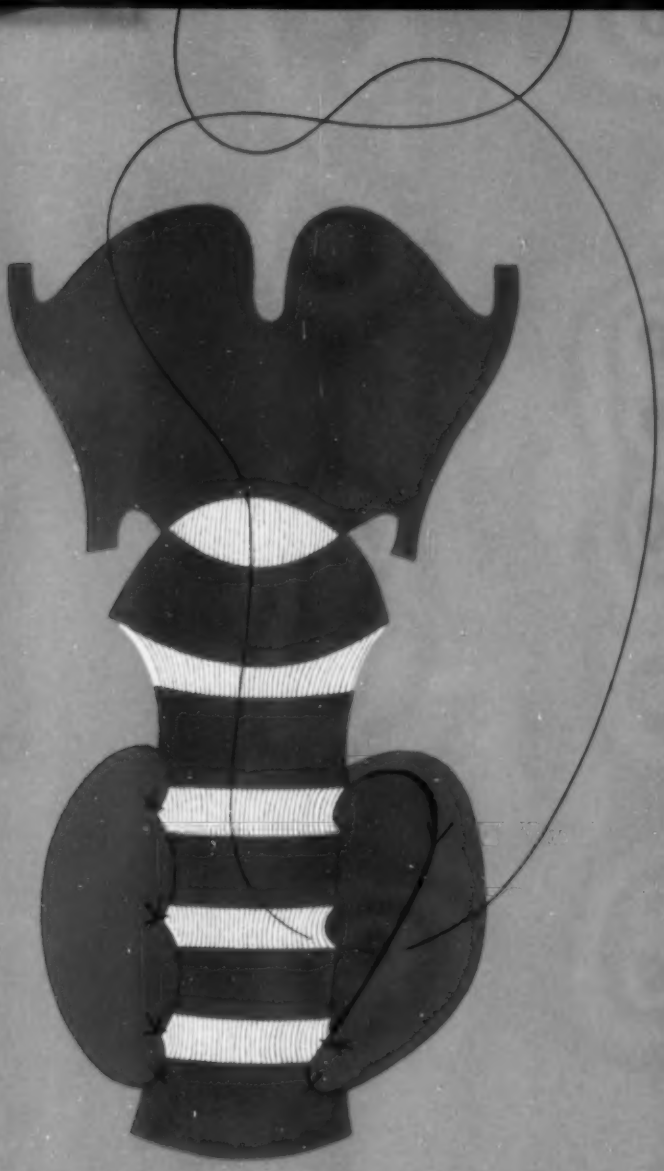

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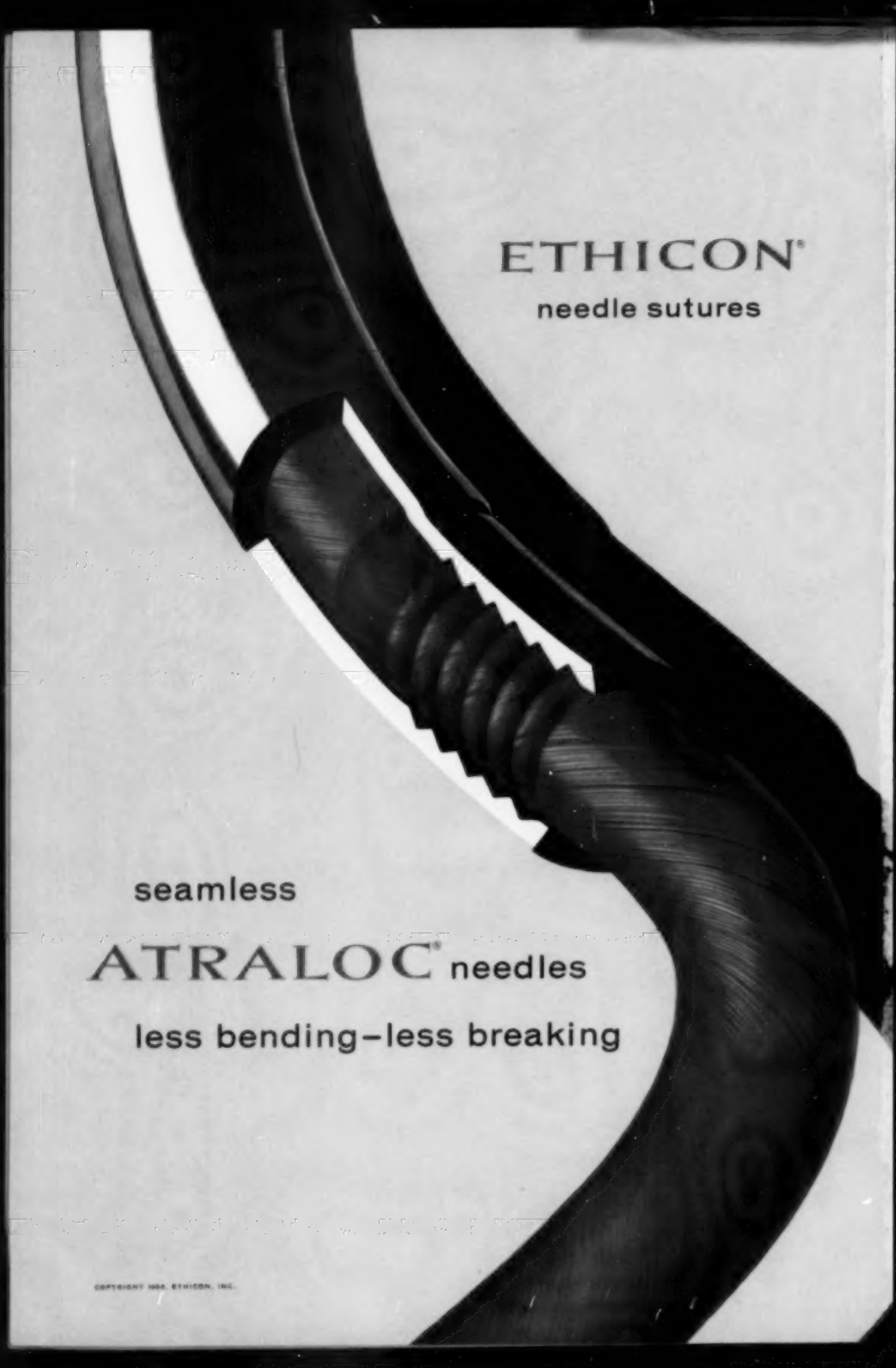

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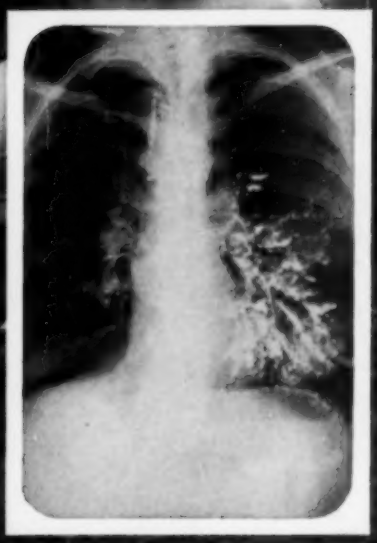

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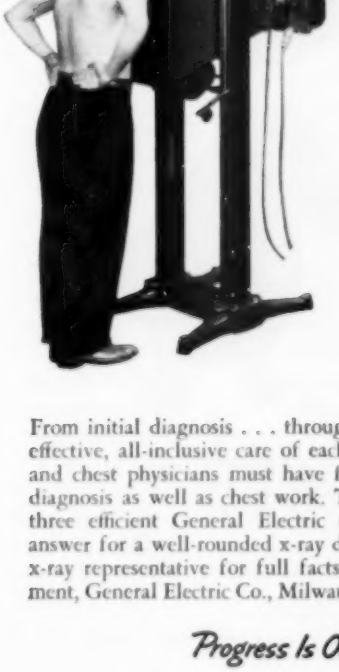

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A HALF CENTURY OF MEDICAL PROGRESS IN THE CONTROL OF TUBERCULOSIS[1]

ESMOND R. LONG

(Received for publication April 26, 1954)

When the twentieth century opened, tuberculosis was the world's most destructive disease. It was endemic in most parts of the earth and spreading in epidemic phase to a few parts previously untouched on civilization's fringe. It took its greatest toll in big cities, where its intensity had been aggravated by the crowding and poverty incident to industrial development. Yet a remarkable phenomenon was under way. Tuberculosis was declining. In this decrease, man's conscious effort had had little part, but the past twenty years had yielded so much new information that a public conscience had developed, and welfare-minded citizens and progressive physicians recognized a responsibility for a direct attack.

In 1904, when the National Tuberculosis Association was organized, a substantial foundation of exact medical knowledge on tuberculosis had been erected. Characteristic anatomical and pathological features had identified it long before as an entity among diseases, and Koch's relatively recent discovery of the causative agent had established its specific infectious nature. The ease of reproducing tuberculosis in animals and the wealth of manipulative laboratory procedures developed by Koch and others for its recognition had dispelled most of the old vagueness on its nature and laid the ghost of fatal hereditary disposition.

The two decades following Koch's discovery were rich in fundamental research on the disease and crowded with practical attempts at its prevention and treatment. The chief staining methods for detecting the tubercle bacillus were developed soon after the germ was discovered; basic investigations on its structure, growth, and composition were carried out shortly afterward, and a large number of efforts were made to fortify human resistance to it. Several antiserums were put forth, all of which ultimately proved of no value, and even before the end of the nineteenth century numerous methods of vaccination,

[1] Presented at the General Meeting on *The People Against Tuberculosis* at the annual meeting of the National Tuberculosis Association, Atlantic City, New Jersey, on May 17, 1954.

based on the smallpox model and experiments of Pasteur, were tested, in which a variety of attenuated and dead forms of the bacillus were used. These early researches are memorable today chiefly because the vaccine, BCG, widely used at present, is a direct descendant of one of them. Calmette and Guérin were among the eminent investigators in this field at the turn of the century, and the attenuated bacillus now called by their names, which had been isolated as a virulent organism by Edmond Nocard in 1902, was already under investigation in 1906 as a possible immunizing agent.

The first decade of the twentieth century was a propitious time for the development of organized effort to fight tuberculosis. New diagnostic methods had become available. X-ray photography, discovered in 1895, was showing promise for practical use in the discovery and delineation of disease in the chest. The tuberculin test, growing out of Koch's not altogether fortunate promotion of tuberculin as a therapeutic agent, became an invaluable tool in diagnosis and epidemiological study when refined in 1907 and 1908 by the methods of Pirquet, Hamburger, and Mantoux.

Vigorous investigations on the chemotherapy of tuberculosis, founded on the successful introduction of salvarsan for syphilis by Ehrlich in 1910, were soon under way. For another twenty years they were to be pursued patiently and persistently along the same lines and then abandoned to await a new time, which actually was not far away, when a fresh approach proved more fruitful. Of this same period were the beginnings of therapeutic pneumothorax, which had been advocated by Forlanini in Italy in 1894 and practiced independently by Murphy in Chicago in 1895.

The time was ripe when the NTA was founded, not only for technical medical advance, but also for progress through social and public health methods. The practice of public health had finally been extended to tuberculosis. Reporting of cases of the disease to an official agency, today recognized as essential in the control of infectious illness, was gradually becoming obligatory. It had been slow in arriving, but the persistence of Flick and Biggs won out over the apathy and not infrequent active resistance of the medical profession. The new National Tuberculosis Association worked vigorously to promote the practice.

A profoundly influential development, dispensary care, was under way. The pioneer stimulus of Robert Philip of Edinburgh in the late eighteen eighties led to the development of city chest clinics throughout the world, and municipal organization against tuberculosis rapidly developed headway. The tuberculosis dispensary, or chest clinic, became the key medical institution in the fight against tuberculosis when integrated as a case-finding and case-referring agency within a sanatorium or tuberculosis hospital system.

The sanatorium movement was gathering momentum in the early years of the century. When the National Tuberculosis Association was founded in 1904, the United States possessed some 9,000 beds for the care of this disease, a pitifully small number in a country with a population of eighty million people, a hundred and fifty thousand deaths a year from tuberculosis, and at least half a million open cases. Most of the 9,000 beds were in private institutions. In the years which followed, state responsibility became widely accepted. Appreciation of

the importance of these institutions in treatment and prevention increased, and the number of beds steadily augmented, reaching a hundred thousand by mid-century. By that time the great majority of them were in municipal, county, state, or federal institutions. By that time, too, the sanatorium in its original sense was passing out of existence. With a change in character of the treatment of tuberculosis, to be described later, it was being converted into or replaced by the tuberculosis hospital. Ideally, it was no longer a rural institution for rest alone, but rather a metropolitan organization for active therapy.

Epidemiological knowledge was fragmentary in 1904, but grew rapidly in succeeding years. The National Tuberculosis Association itself placed much emphasis on the tuberculosis mortality rate, interest in which for years overshadowed concern with the more important rate of prevalence of the disease. The latter was first investigated in a substantial way through a combination of effort by the Metropolitan Life Insurance Company and National Tuberculosis Association in 1928 in the famous Framingham demonstration, which indicated that the prevalence of active and potentially active cases was approximately ten times the figure for the annual rate of death from the disease. This figure was used for years, and is only now giving way to a more realistic formula.

When the NTA was organized, professional knowledge among practicing physicians on the nature and prognosis of the disease was woefully deficient, not yet out of the folklore tradition. With many, the treatment was still the reverse of that most desirable. Exhausted tuberculous patients, needing rest and quiet more than anything else, were driven to hard, outdoor jobs. Almost the only doctors with rational ideas on tuberculosis were those who had had the disease themselves. At the time, forty to fifty years ago, they were the only ones sufficiently impressed by the disease to specialize in it.

Their education, however, had been neglected. There was little systematic instruction on tuberculosis in medical schools. Then, and for years afterward in this country, study of the disease that killed more people than any other was only an elective in the medical school curriculum, and not many elected to take it.

Doctors found few cases of tuberculosis in its early stages and sent their patients, when they could, to distant sanatoriums for the more expert care of physicians who had had the disease themselves. Sanatorium doctors, in the tradition developed by Brehmer, Dettweiler, and Trudeau, stressed rest and plenty of food as the means to get well. In spite of high death rates, optimism and morale were conspicuous among sanatorium patients.

There was little appreciation by the medical profession of 1904 that the high prevalence of tuberculous infection was avoidable and in itself the main target for attack. The most erudite physicians took refuge in the view that "everyone had a little tuberculosis," and consoled their patients accordingly. Necropsies in general hospitals did indeed show signs of old tuberculosis in most human bodies, and the overwhelming majority of adults living in cities reacted to tuberculin. It is difficult for physicians who were not part of the period to appreciate the profound influence of the theory of universality of tuberculous infection on concepts of tuberculosis and medical practice at that time.

DEVELOPMENT OF KNOWLEDGE

A remarkable growth of understanding took place in the first quarter of the century. The medical literature of the period was intensely concerned with the relations and significance of allergy resulting from infection and the phenomenon of acquired resistance to tuberculosis. Thinking was inextricably bound with the concept of universal infection, and the acquisition of allergy without the dangers of infection was a goal persistently sought. In a day when most children were infected and the view prevailed that adult tuberculosis was endogenous, infection in childhood was deplored because it set the stage for recrudescence of the disease from the latent lesions of first infection within the body, and at the same time was considered a fortunate provision of nature because it set up resistance to later infection from without.

Although most children then as now recovered from primary infection, the progressive forms of first infection in children were terrifying, and those who believed in the malignancy of primary infection found their views confirmed by the extreme gravity of tuberculosis in races not exposed to the disease in childhood. Some of the puzzle has since been solved through better understanding of native resistance and the phenomenon of natural selection in populations. It has finally become understood that first infection led to such severe tuberculosis in races of people previously unexposed, not because it was first infection, but because the susceptible stock in those races had not been removed in the process of survival of the most fit, as had been accomplished by centuries of exposure in the white race. The release from fear of primary infection today, when it is seen with decreasing frequency in childhood, and equally of fear of devastating epidemics in a population that escaped primary infection in the early years of life, is a satisfaction not anticipated in the early days of the NTA.

Argument over the role of childhood infection restimulated the age-old search for a vaccinating agent, and the number and variety of vaccines proposed became fantastic. Few people today realize the extraordinary extent of the vaccination endeavor. In general, the many proposed vaccines have been of three main types: (*1*) tubercle bacilli attenuated by adverse conditions of growth, (*2*) bacilli killed by means calculated to destroy their power to invade animal tissues while conserving the capacity of their constituent parts to induce immunity, and (*3*) biological variants of those tubercle bacilli whose natural habitat was in another environment. The ingenuity of these attempts was extraordinary, and the remarkable fact is that, in the hundreds of reports on the vaccinating agents tested, some degree of success was nearly always claimed. The undeniable partial success attained has kept the search alive and active, and to some extent has pointed the way for new efforts. Modern basic studies on the nature of resistance to tuberculosis have in the meantime bridged some of the gap between native and acquired immunity and opened a few promising leads on the enhancement of human resistance. There is reason to believe that their pursuit will take investigators along quite different paths, however, from those followed in the conventional types of vaccination.

No account of the force of modern medicine in the fight against tuberculosis should fail to record the influence of scientific media for the development and dissemination of knowledge. It is trite but none the less true that research stimulates research. The tuberculosis movement has profited from the devotion of substantial funds for investigation not only by special institutions set up for the study and treatment of the disease, but also by schools of medicine, wealthy philanthropic organizations, and official governmental agencies.

The National Tuberculosis Association takes pride in two important developments of its own in this field: the creation in 1917 of a technical journal for publication of new information on tuberculosis, THE AMERICAN REVIEW OF TUBERCULOSIS, and the establishment in 1921 of a tuberculosis research program. Both were among the first ventures of this type by voluntary agencies, and it is not too much to say that each has been a model and a stimulus to other organizations.

Throughout the years, and in greater measure recently, research on tuberculosis has been strengthened by intensive investigations in sister sciences, particularly in organic chemistry and pharmacology. The net result, as we shall see in a few moments, has been a series of valuable drugs for the treatment of the disease.

PREVENTION OF TUBERCULOSIS

A sharp advance in the prevention of tuberculosis took place in the nineteen twenties and thirties when persistent efforts initiated by the Bureau of Animal Industry virtually eradicated bovine tuberculosis in this country, a disease that had long been a source of crippling infection for children. The basis for the campaign was the tuberculin test and slaughter of infected herds.

The analogy in man of the radical campaign in cattle was the discovery and isolation of infective cases. Case-finding programs by chest clinics, systematic supervision of contacts, and mass surveys for the discovery of cases among the apparently well have together removed hundreds of thousands of infective cases from communities, and are in large measure responsible for the great decrease that has occurred in the prevalence of childhood infection. Group surveys, now widely practiced in schools and industry, and mass surveys of whole cities by the photofluorographic method of Abreu have set up a model procedure for the future.

Isolation of cases has been of immense effect in diminishing sources of contagion but has not always been easy, particularly among patients who feel entirely well. Many communities have taken the drastic step of forcible isolation of incorrigibly careless patients with open contagious disease. Complete isolation fortunately is ordinarily not necessary. Proper medical supervision and intelligent cooperation by the patient go a long way in preventing dissemination of infection.

As noted previously, attempts to prevent tuberculosis by artificial immunization have been numerous and persistent. Mention has been made of early efforts at passive immunization by antiserums. They failed, and more recent

experiments with the gamma globulin of antituberculosis serums have not been more successful.

On the other hand, in spite of a discouraging lack of success with the overwhelming majority of the numerous agents studied, measurable, if not decisive, success has attended the effort to immunize actively against tuberculosis by vaccination. Of the large number of products tried with more or less rationale, one practical vaccine, BCG, has survived. There is no question that it raises resistance to tuberculosis in some degree. The World Health Organization, following the lead of French and Scandinavian investigators, has adopted the point of view that the measure of resistance conferred by BCG is of significant value when the procedure is carried out in conjunction with other established methods of tuberculosis control. On that basis the organization has supervised and stimulated the prophylactic treatment of millions of persons. Good analytic research is needed, and fortunately is in prospect, to evaluate the results.

MEDICAL AND SURGICAL TREATMENT OF TUBERCULOSIS

For thirty years, from 1915 to 1945, the most widely practiced specific treatment for tuberculosis was artificial pneumothorax. So convinced of its value were sanatorium physicians that at the height of its popularity it was not uncommon to find 90 per cent of the patients in an institution under treatment by this method. With the advent of chemotherapy, its defects, although long appreciated, became conspicuous, and its use declined greatly. Its exact place in present treatment is debated, but, since antimicrobial and surgical therapy have become so well developed, artificial pneumothorax has become an accessory rather than a primary procedure.

The rise of chemotherapy has been the most dramatic event in the history of tuberculosis. Almost abandoned after failure of success on the salvarsan model, chemotherapy came to life again with the development of the sulfa drugs in the middle nineteen thirties. The success of sulfanilamide in the treatment of certain acute infections paved the way. Several synthetic sulfur-containing antimicrobials achieved some success in tuberculosis. They were, in general, too toxic in the required dosage, however, and were generally discarded after the discovery of the antibiotic streptomycin in 1944. As in the case of the sulfa drugs, success in other diseases had opened a new path. Streptomycin became to tuberculosis what penicillin was to acute suppurative and septicemic infections.

Streptomycin, however, proved to have some toxic potentialities, and its use suffered from the additional defect of bacterial tolerance after its prolonged employment. Reduction in dosage and combined therapy with para-aminosalicylic acid (PAS) obviated these difficulties in large measure, and highly effective regimens were developed, with beneficial results never seen before in acute forms of progressive tuberculosis and the chronic pulmonary disease as well.

The combined or associated therapy of tuberculosis with streptomycin and PAS was hardly established when the world was startled by the discovery of high antituberculous activity in certain aromatic chemical compounds, some of which were synthesized long ago. The best known and most effective of these

for practical use is isonicotinic acid hydrazide, which appeared under several trade names and has since been officially designated for brevity as isoniazid. It is almost nontoxic and has been shown by exhaustive tests to be of essentially the same order of therapeutic efficacy as streptomycin. But the quest is not yet over, and other drugs, notably pyrazinamide at the present writing, are strong competitors of streptomycin, PAS, and isoniazid.

Coincidentally with chemotherapy, thoracic surgery has been progressively developed. From rough beginnings in which drastic reductions of the size of the rib cage were induced in cases in which pneumothorax had failed or was impossible, a series of refinements in technique were effected and with such success that, before the advent of chemotherapy, primary thoracoplasty was fast replacing pneumothorax. With gathering skill and improvements in technique, thoracoplasty has become a selective procedure adjustable to anatomical needs, causing relatively little visible deformity of the chest wall.

Commencing at about the time of the discovery of streptomycin, a bold development of excisional surgery for tuberculosis took place. It was at first modeled on a relatively new excisional surgery for cancer and extensive suppurative disease of the lungs and limited to the grosser divisions of the respiratory tree, i. e., whole lungs or their major divisions, the pulmonary lobes. Later, excisional surgery for tuberculosis proceeded to finer and finer subdivisions, until at the present time it is common to remove bronchial segments for localized disease and extract individual tuberculous lesions in simple wedge resections of lung tissue. In all of this, the meticulous training of young, skillful surgeons, great improvement in anesthesia, associated chemotherapy, and careful study of lung capacity before and after operation by methods devised for the study of pulmonary function have been of inestimable value.

Today, the standard treatment of advanced tuberculosis is essentially a combination of drug therapy and thoracic surgery, the former controlling the inflammatory elements of the disease while the latter removes tissue irreparably damaged. Treatment has come a long way from the passive rest and hygienic care of 1904, and a long way too from the active regimen of pneumothorax which dominated tuberculosis therapy two and three decades later.

SUMMARY

Altogether, in the fifty years since the founding of the National Tuberculosis Association, an extraordinary growth of medical knowledge on tuberculosis has taken place and fortunate developments in the control of other microbial diseases have been capitalized in the treatment of tuberculous disease. An effective combination of drug therapy and thoracic surgery has revolutionized the treatment of tuberculosis within the last decade.

In the laboratory, long and patient investigations have laid bare the natural history of the remarkable germ causing tuberculosis and gone far in developing an understanding of the physiological elements in the human body responsible for susceptibility and resistance.

Practical methods of prevention of the disease, represented chiefly in case

finding, isolation of infective cases, and supervision of contacts, have greatly lowered the spread of infection, supplementing powerful social forces which have long operated in the same direction. An immunizing agent, reasonably effective in conjunction with other measures, BCG, affords protection to uninfected persons likely to be unduly exposed.

Many leads for future progress have been uncovered. Chance and quick development may be expected to facilitate new advances in tuberculosis control. For the long run, however, it is still essential to stimulate young investigators for the future, and promote constructive scholarship in advancing fundamental understanding of still obscure facets of the disease in order to guarantee uninterrupted further progress.

UNSOLVED PROBLEMS IN TUBERCULOSIS[1]

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Tuberculosis occupies a peculiar place in the history of medicine. By the end of the nineteenth century, more was known of its clinical manifestations and pathological characteristics than was the case for any other important infectious disease. Yet, the genesis of its lesions and the factors which influence their course constitute today one of the most obscure chapters of pathology. Similarly, while the tubercle bacillus was one of the first pathogenic microorganisms to be isolated and extensively studied both *in vivo* and *in vitro*, knowledge of its pathogenic determinants has hardly advanced during the past two decades. Factual information concerning its metabolism and composition, its immunological behavior, and the properties responsible for its virulence is so deficient that it contributes but little to the understanding of the disease. In other words, the study of tuberculosis, which was in the forefront of medical sciences fifty years ago, now lags several decades behind that of many other human infections.

This paradox in the evolution of knowledge is reflected in the history of the antituberculosis campaigns. Enough was known in 1900 to permit the formulation of a broad control program embracing, not only the care of the tuberculous patient, but also preventive public health measures and social policies aimed at the prevention of tuberculosis. For the first time, a concerted attack—carried simultaneously along scientific, medical, and social lines—was being organized against a particular disease. This fact, of enormous historical significance, bears witness to the genius and breadth of vision of the pioneers of the antituberculosis movement. There is reason to believe, however, that, if the manifestos which heralded the movement all over the world sound so amazingly modern today, it is in part because the past few decades have added but little objective knowledge, and imaginative vision, to carry the problem of tuberculosis beyond the stage that it had reached half a century ago. Indeed, it is not unlikely that the very thoroughness and comprehensiveness of the program formulated at the beginning of this century account in part for the slackening of research. In contrast to what obtained for other diseases, tuberculosis workers became committed to a number of well-defined and very practical policies which monopolized their creative thoughts and energy. It is clear in any case that the antituberculosis movements presently live to a very large extent on the scientific wealth inherited from the late nineteenth century.

Needless to say, the enlightened approach based on early theoretical information has resulted in great practical achievements, but it is equally true, never-

[1] Based on addresses delivered in Denver on March 12, 1954, at the dedication of the Gerald B. Webb Memorial Building, Colorado Foundation for Research in Tuberculosis; and in Atlantic City on May 21, 1954, at the celebration of the Fiftieth Anniversary of the National Tuberculosis Association.

theless, that tuberculosis is still with us. Indeed, now that techniques are available to prevent or cure most of the other great plagues of the past, tuberculosis constitutes by far the most important infectious disease of man in the Western World—and I am afraid that it is likely to remain a very large problem for many decades unless a new vision and new knowledge are brought to bear on it.

You will, of course, question the validity of these pessimistic statements at a time when drugs and surgery are revolutionizing the treatment of tuberculosis. Do we not read in the lay press and in scientific articles that the conquest of the White Plague is now in sight? To any one conversant with the history of the disease, this affirmation has a familiar sound and is indeed somewhat painful. It has been made so often in the past, in ignorance of the complexities of the problem! I could contribute a rich source of documents to the history of human illusions by reciting before you many of these optimistic forecasts. Instead, I shall limit myself to one, which has a certain piquancy on this particular occasion:

> One may unhesitatingly say that the child is now born who will live to see the year in which a well-sprinkled, well-swept, well-watched, and well-sanatoriumed city will put as many difficulties in the way of the frequent local study of tuberculosis as now exist for the local study of smallpox or typhoid.

This, you probably believe, is a quote taken from a recent speech, the product of intellectual intoxication and wishful thinking produced by one of the new therapeutic or public health procedures. Not at all. It was said in 1905 by Talcott Williams in one of the welcome addresses at the very first meeting of this Association. Tuberculosis was even then on its way out. It has been ever since.

The reason for the periodic bursts of optimism is, of course, plain to see. The mortality of tuberculosis has been following a downward course for almost a century, and there is reason to hope that this trend will continue. It is tempting, moreover, to attribute to each new therapeutic or preventive measure a determinant influence on this phenomenon, and to see in it the weapon that will finally wipe out the disease. In reality, however, it is only because we continue to think in nineteenth-century terms that we can listen without irritation to statements that tuberculosis has been conquered. At the time when antituberculosis campaigns were being organized, attention was focused on the appallingly high numbers of tuberculous deaths. It was natural, therefore, to use the decrease in mortality as a criterion to measure the efficacy of control programs. The results are known to everyone. Tuberculosis is no longer one of the leading causes of death in this country; in fact, it has not been for more than a decade. True enough, tuberculosis still kills more people than any other microbial disease —evidence that it has not been conquered—but the death rate has been reduced to a level sufficiently low to remove much of the fear that it once inspired. There are indications that the public in the United States is willing to settle for a tuberculosis mortality of some 20,000 a year.

Mortality, however, is not the real yardstick to measure the importance, or judge of the control, of a disease. Even if there were drugs capable of preventing the sudden deaths resulting from hypertension and arteriosclerosis, these condi-

tions would remain a huge medical and social problem. Similarly, adults do not commonly die of mental disorders, arthritis, or peptic ulcers. Yet, no one would claim that these afflictions have been conquered. Neither has tuberculosis been conquered. Instead, the forces which have been at work during the past century have slowly converted it from a killing to a chronic disease. This is, of course, a great achievement, but its distant consequences may well generate new problems. By handicapping their victims without killing them, chronic diseases have come to constitute a huge financial and emotional problem—ever increasing in size—which threatens to choke the medical economy of our communities. It is folly to regard them as solved medically until they have been *eradicated*.

Before turning to some of the technical aspects of the eradication of tuberculosis, I must apologize for presenting my argument in the form of dogmatic statements. On the one hand, lack of time will prevent the discussion of evidence. I hasten to acknowledge furthermore that factual information is so inadequate in most cases that it can at best warrant opinions, but certainly does not justify absolute beliefs. As a result, I shall often express views which are not shared by experienced physicians and students of tuberculosis. Let me note in passing how the very fact that earnest and disinterested seekers for truth can hold profoundly divergent views constitutes telling evidence for the need of intensive research on the intimate nature of the disease. It would be easy and pleasant to belittle these conflicts of opinion and to deal only with the noncontroversial issues. Instead, I have chosen the harder task to discuss before you certain areas of disagreement and ignorance, and to emphasize in particular that we have failed to solve many of the theoretical and practical problems formulated by our predecessors fifty years ago. To guide my selection of topics, I shall resort to the address presented in Washington by E. L. Trudeau in 1905, when, in his capacity as first president of the National Association for the Study and Prevention of Tuberculosis, he discussed what he regarded as the most important "unsolved problems of tuberculosis."

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It is obvious that the most direct approach to the eradication of tuberculosis is to prevent tubercle bacilli from reaching human beings. For this reason, Trudeau logically put first on his list of problems "the study of different sources of infection."

Now that bovine tuberculosis has been all but wiped out in this country, man himself is the source of practically all infection. Even though so sharply limited, the problem remains, nevertheless, ill defined. It is generally agreed that there are at the present time in this country approximately 350,000 persons suffering from active tuberculosis and 800,000 showing pulmonary lesions probably referable to tubercle bacilli. These figures, however, have meaning only in terms of the effectiveness of the diagnostic procedures which were used in obtaining them. Many persons are listed as tuberculous today who would have escaped diagnosis before the days of refined roentgenography. On the other hand, it is certain that small necrotic lesions of much potential danger remain undetected by the most thorough medical examination. It can be accepted as a fact that infection with

tubercle bacilli commonly results in necrotic lesions which—even though not ordinarily detectable—persist and remain infected for a long period of time. Since tuberculin surveys clearly show that a very large percentage of adults have been infected, it is prudent to assume that many of them still carry virulent bacilli.

In this light, it appears probable that tuberculosis often takes the inapparent form described under the name of latent infection in many other microbial diseases. The analogy with fever blisters caused by the herpes simplex virus immediately comes to mind. The herpetic person usually becomes infected during the early years of life and continues to carry the virus without being disturbed by it under normal circumstances. However, the presence of the virus is revealed by two different types of manifestations. On the one hand, it elicits a high degree of immunity to reinfection with virus introduced from the outside (as does primary tuberculous infection against exogenous reinfection). On the other hand, the latent virus infection can be "provoked" into activity by a large variety of unrelated stimuli (fevers, menstruation, nutritional disturbances, irradiation, et cetera), thus giving rise to self-limiting disease of the fever-blister type. Is it not likely that latent tuberculous infections can also be "provoked" into activity by various types of physiological disturbances? There is, as yet, no convincing experimental evidence in this regard, but clinical experience clearly points to many situations in which the appearance of an active tuberculous process might be explained in such terms.

It is my conviction that many aspects of the pathogenesis and epidemiology of tuberculosis will remain obscure until more is learned of the mechanisms capable of converting inapparent infections into active disease. In reality, this field of research should be considered as a new phase of the etiology of tuberculosis—that concerned with the environmental and physiological factors which affect the relationship between the infective agent and the host cell. In brief, it appears likely that many millions of persons carry in a latent form living, virulent bacilli. On the records of today are listed only the patients with obvious symptoms, or with lesions gross enough to be visible by our techniques of observation. On the records of tomorrow, there will appear in addition those in whom the dormant lesion has been rendered active as a result of decrease in general resistance. There is no sharp differentiation between infection and disease. The criteria which separate one from the other are determined in no small part by the acuteness and theories of the physician and by the social mores of the time. If there is anything true in this theory, there exists all over the world—and even in this country—an enormous and unchartered reservoir of bacilli. We shall now consider its possible importance for the spread of infection.

As we all know, one of the cardinal features of the antituberculosis campaigns all over the world since 1900 has been to search for the "sputum-positive" persons, segregate them if possible, and, in particular, educate them to the potential danger which they constitute for their fellow men. The success of this program is apparent in the fact that a large percentage of young people in this country remain tuberculin negative throughout their school years. In my opin-

ion, this is the greatest single practical achievement to date in the fight against tuberculosis. But it is also true that very large numbers of adults eventually become tuberculin positive, proof that they come in contact and are infected with virulent bacilli. To repeat in another form Trudeau's query: Where are the spreaders of bacilli? How can they infect so many of their fellow men? The usual view is that they are found among the persons suffering from active tuberculosis, some 350,000 persons in this country. Not all of these, however, are "sputum positive," and many are segregated in hospitals or sanatoriums. Is it certain that the fairly small number who are at large and expectorating bacilli can account for the rapid rate at which young adults become infected after they are removed from the sheltered environment provided by the school? Is it not worth considering that, among persons who give no evidence of active tuberculosis, some may at times become transient spreaders of bacilli and contribute to the spread of infection? The question is of enormous theoretical and practical importance, involving as we have seen the possibility of a latent form of tuberculosis susceptible of activation by various forms of stress.

Unfortunately, the diagnostic methods now available do not lend themselves to the study of this problem. The primitive bacteriological techniques worked out decades ago for the detection of bacilli in sputum and other pathological materials are still in use today. Yet, it is certain that most bacilli do not survive the barbarous treatments with alkali or acids to which they are submitted during the preparation of the specimens for the culture tests. Moreover, it is also beyond doubt that the empiricism symbolized by the use of egg and serum media constitutes only a crude approximation to the conditions which are optimal for bacillary growth.

Nor are cultural procedures the only deficient links in the diagnosis of tuberculosis. The serology of the disease is all but nonexistent, consisting of a few crude reactions with products of old and autolyzed cultures, ill-defined in chemical composition and in their relation to the structure and pathogenic properties of the bacilli. As to the time-honored tuberculin reaction, it is still in concept and practice the same as when first observed by Koch and used by von Pirquet. Millions upon millions of tuberculin tests are carried out every year all over the world, but fundamental studies of the significance of the reaction are very scarce. What is the nature of the active component of tuberculin? How specific is the allergy which it detects as an index of tuberculous infection? Does the allergic state persist after disappearance of the bacilli; and for how long? How is it influenced by the physiological state of the person? What is the relevance of the test to antituberculous immunity? For lack of definite factual knowledge concerning these fundamental questions—and many others—personal opinions hold sway, and it is not surprising that such divergent interpretations can be placed on the results of tuberculin surveys. Trudeau was well aware of the importance of the test as a diagnostic tool, and he begged for "more exact knowledge of its scope, its limitations, and its real value."

In brief, full understanding of the epidemiology of tuberculosis demands a more dynamic concept of the relation of infection to disease. Its study would be

facilitated if there were available convenient and quantitative bacteriological and immunological procedures which would permit following the fate of the bacilli and the tissue response which they elicit under conditions of latency or pathological activity.

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In his presidential address, Trudeau expressed particular interest in "the possibility of producing artificial immunity to the germ or to its toxins." His own experimental work, and that of his contemporaries, gave reason to believe that effective methods of vaccination could eventually be developed. Indeed, it was then well known that it is possible to enhance the resistance of experimental animals to tuberculosis by the use of vaccines consisting of either attenuated living bacilli or suspensions of killed organisms. These two techniques of vaccination still have their advocates today. Moreover, surprisingly little has been added to them in theory or in practice. In this respect, it is of interest to quote the conclusions derived by Trudeau himself from some experiments which he reported before the first meeting of this Association:

> . . . dead tubercle bacilli increase, though to a very slight degree, the animal's resistance to subsequent inoculation.
>
> . . . the living attenuated bacillus gives a stronger degree of immunity than the same bacillus killed by heat.
>
> . . . the degree of attenuation of the bacillus used as vaccine bears a distinct relation to the degree of protection it affords in guinea pigs to subsequent inoculation with virulent human cultures.
>
> . . . cultures derived from cold-blooded animals and which only grow at room temperature . . . have brought about no appreciable degree of immunity.

It is a remarkable, but also somewhat humiliating, fact that little need be changed or can be added to these conclusions today.

It is also true, furthermore, that the very same objections which have limited the usefulness of antituberculous vaccination in the past still stand in the way of its general adoption today. Among many difficulties which could be mentioned is the lack of valid techniques for biological standardization of the various vaccines and for measuring the level and duration of the immunity to which they give rise. It is disturbing also that the vaccines presently available render the vaccinated person tuberculin positive and thereby deprive the physician and the epidemiologist of a diagnostic test which is becoming increasingly useful as the average age of primary infection advances. This, of course, would be of little importance if the vaccines did provide an absolute protection against tuberculosis. Unfortunately, it is certain that the degree of immunity that they induce is of a low order. In fact, there is no evidence that this immunity can ever prevent superinfection; it only retards its course. Vaccination may, therefore, be of value against the acute forms of the disease, such as miliary and meningeal tuberculosis, but in its present form it can hardly be very effective in solving the far greater social problem of pulmonary tuberculosis.

Thus, while experimentation in animals suggests the possibility of increasing

resistance to tuberculosis by vaccination, it is clear that the promise is still far from ideal fulfillment. Indeed, it is probable that little progress can be expected in this area until something has been learned of the specific bacillary constituent capable of eliciting protective immunity and of the nature of the tissue reaction resulting in the protective effect. Precise understanding of the mechanisms of antituberculous immunity transcends in importance its application to the development of a more effective vaccine. For this knowledge might lead to the formulation on a rational basis of objective tests to determine the state of immunological resistance of an individual. As is well known, conflicting views are held by different students of tuberculosis on the importance of acquired immunity in this disease—some giving it a role of paramount importance, others denying that it has any significant protective value. Here again, the very existence of these controversies reveals the inadequacy of our knowledge. There is an urgent need for a more exacting analysis of the immunological processes elicited by tuberculous infection.

While there is profound disagreement concerning the importance of acquired immunity, no one questions the importance of natural resistance in tuberculosis. On the one hand, it is clear that each individual possesses innate characteristics which determine the manner and intensity of his tissue response to the presence of tubercle bacilli. On the other hand, clinical observations reveal that physical and mental fatigue, metabolic disorders like diabetes and starvation, and many other nonspecific physiological disturbances, often undermine resistance—while good living increases it. All this was well known fifty years ago and, among the problems of which he urged the study, Trudeau listed "above all, the mechanism of natural immunity."

The problem of natural immunity is still unsolved and, unfortunately, little has been added to the understanding of it possessed by Trudeau's contemporaries. Yet, its importance looms larger every day. It is obvious that, of the millions of persons who become infected with tubercle bacilli, only a small percentage develop clinical disease. Clearly, the determinants of disease are not the same as the determinants of infection. If the factors capable of provoking infection into activity could be recognized and measured, much disease could certainly be prevented and the management of the patient could become less empirical. Resistance and susceptibility are not abstract attributes. They are the outcome of chemical and physiological reactions between tissues and bacilli, and these reactions are amenable to experimental analysis. But the study of this problem demands fresh points of view. Anyone who knows how to observe and to think has a chance to contribute to it, if he can recognize in each patient, not just another case of tuberculosis, but the play of forces which succeeded at a given time in breaking down the resistance which human beings normally possess against tubercle bacilli.

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Chemotherapy and resectional surgery occupy today the center of the stage in tuberculosis because, needless to say, it is in these areas that the most spectacular advances have been made during the last decade. If measured by the

numbers of lives saved or at least extended, the effects of the new therapeutic procedures are striking indeed. But it is unfortunately much harder to evaluate their results in terms of the influence which they are likely to exert on the social aspects of tuberculosis and its eventual eradication.

To comprehend the shortcomings of the present therapeutic practices in tuberculosis, it is useful to compare their results with those obtained in the treatment of streptococcal or pneumococcal infections, gonorrhea, or syphilis. It is possible in these diseases to completely rid the body of the infective microorganisms by adequate administration of the proper drug. In contrast, this ideal goal of therapy is very hard to reach in pulmonary tuberculosis with the drugs presently available. Even when chemotherapy and resectional surgery succeed in rendering the tuberculous process completely inactive, they commonly fail to achieve bacteriological cure. The difficulties which stand in the way of this goal cannot be discussed in detail here, but it seems useful to present them in broad outline.

In vivo, tubercle bacilli exist in many different types of environment: intracellularly within various types of cells, extracellularly exposed to the great variety of physicochemical conditions determined by the nature of the inflammatory response and by the extent and kind of tissue necrosis. The protean character of the infectious process thus causes bacilli and drugs to come into contact in areas differing markedly in physicochemical characteristics, often isolated one from the other, and in which the prevailing conditions antagonize to a different degree the antimicrobial activity of the various drugs. As drugs are selected on the basis of their ability to function in the normal physiological environment, they are often ineffective within the lesions, where conditions are profoundly different from the physiological. It is this fact which is responsible for the difficulty in achieving complete eradication of the bacilli in pulmonary tuberculosis. Let me mention in passing that it accounts also for the failure to prevent the emergence of drug-resistant forms of the bacilli, even when several different drugs are used simultaneously. On the other hand, only in rare cases can resectional surgery achieve complete removal of all infected tissues, for living bacilli exist in many small lesions which are beyond the reach of the surgeon. In final analysis, it is the body itself which, through the agency of its own antimicrobial defenses, must check the growth of the bacilli that have survived the onslaught of drugs and surgery. Unfortunately, little is known indeed of the nature and effectiveness of these natural defense mechanisms.

The limitations of the present therapeutic procedures call for a renewed study of the pathology of tuberculosis, with particular regard to the localization of bacilli in the various normal and pathological structures and to the effect of tissue factors on their survival and multiplication. It is well possible that added knowledge in this field would permit the development of new screening tests leading to the discovery of other kinds of drugs capable of attacking the bacilli within necrotic lesions and to more pointed surgical intervention. But, in the meantime, we must reconcile ourselves to the fact that the present techniques of therapy rarely lead to bacteriological cure. While their systematic use may

slow down the rate of infection, it cannot be counted upon to bring about the eradication of tuberculosis in the near future.

Tuberculosis mortality was decreasing at a rapid rate before the introduction of antimicrobial therapy and resectional surgery. We can confidently expect that the downward trend will be somewhat accelerated by these therapeutic procedures, but their very success is likely to add to the size of the chronic disease problem in tuberculosis. As many of the persons saved from tuberculous death will continue to harbor living bacilli, it is wise to anticipate that we shall have, for several decades at least, a large population in constant need of medical supervision to ward against the possibility of reactivation of disease and of further spread of infection. This situation will put additional stress on public health and medical services, and renders even more urgent the need for improved diagnostic techniques—bacteriological, immunological, and clinical. As we have already seen, much remains to be done in this field.

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To prevent death from tuberculosis was a sufficient ideal for the beginning of the antituberculosis movement. But each generation must accept new responsibilities. Ours is to prevent tuberculosis from continuing to cause the waste of life and destruction of human values in those who suffer but do not die of it. In other words, it is time that we escape from the limitation of nineteenth-century concepts and give to the expressions "control" or "conquest" of a particular disease a meaning consonant with the needs and possibilities of our time. It will be justified to speak of the conquest of tuberculosis only when at least one of three goals has been reached: to stop completely the spread of infection, to eradicate the bacilli from the infected person, or to prevent infection from ever expressing itself in the form of lesions and symptoms.

No possibility of real control is as yet in sight in the case of most chronic diseases. The situation is different with regard to tuberculosis. True enough there is no foolproof therapeutic procedure which can be counted upon to achieve its cure. Furthermore, it is not yet practically possible to put into effect in many communities the theoretical knowledge which would permit its eradication by preventing the spread of bacilli. But, on the other hand, it is known that tuberculosis is not a necessary accompaniment of human life. It was a problem of negligible importance in certain primitive societies, and, more important, a few civilized communities in which it was once a scourge have made themselves almost free of it. There is no doubt, therefore, that human tuberculosis can *really* be conquered. Indeed, there are several independent approaches to its prevention and eradication.

We can legitimately hope that there will be discovered less cumbersome and more refined diagnostic techniques, a vaccine free of gross objections, more pointed surgical procedures, and drugs which are eradicative instead of merely suppressive. We know for a fact that it is possible to minimize and even prevent infection. This has been achieved in several communities in this country and in Europe merely by expending sufficient effort and strict social discipline in the application of well-tested public health and medical policies. Finally, it is ob-

vious that the general state of physiological health has much to do with resistance to infection. History has proved time and time again that tuberculosis recedes wherever social and economic conditions assure for man an environment conducive to physical well-being and happiness. It has often been pointed out that the mortality caused by tuberculosis—and by other microbial diseases as well—had begun to decrease before the campaigns based on the germ theory had been put into effect. In reality, however, the campaign against infection began with the great public health reforms of the eighteen thirties. True enough, the early pioneers of the public health movement were little concerned with germs, but they knew that an effective way to combat consumption and other "infectious fevers" was to assure for each citizen good air, pure water, adequate food, and pleasant and happy surroundings, both at work and at play. The first important manifestation of this philosophy was the organization in England of "The Health of Towns Association," the prototype of the present-day voluntary health movements throughout the world. Thus, one can recognize an unbroken continuity of effort, beginning with the social and sanitary reforms of the early nineteenth century and culminating in the more specialized work of the antituberculosis association. A great lesson can be learned from this effort. Most infectious diseases, including tuberculosis, decrease in prevalence and severity as the standard of living improves, and they cannot be fought effectively in industrialized and urbanized civilizations if other aspects of health are neglected.

In the light of these facts, antituberculosis associations appear in a dual role which assures them a unique place in the history of human societies. By hunting the tubercle bacillus wherever it reveals its presence—in latent infections as well as in overt disease—they have within their power to bring to completion the specific task which they undertook fifty years ago. They can demonstrate that it is possible by applying scientific knowledge and social discipline to eradicate a disease heretofore accepted as an unavoidable fate. By advocating habits and behavior which make man less vulnerable to tuberculosis, they can lead the community to a way of life that makes for general well-being and increased resistance to most infections. To treat the sick is the tradition of classical medicine. To aim at the prevention of tuberculosis was a new concept when introduced half a century ago. To visualize its eradication through a betterment of human life really opens visions of a new social order.

The internal logic of the antituberculosis movement demands that it progressively evolve into a health movement. This, however, will impose a searching analysis of medico-social concepts. For health is not easy to define. It involves all aspects of human nature and their relation to the physical and social environment. The history of medical thought during the past 150 years brings to light a striking evolution of medical philosophy in this regard. The nineteenth century reformers considered poverty and filth as the major causes of disease. They believed that pure air, pure water, pure food, and life as close as possible to the ways of nature would correct the evils generated by the Industrial Revolution and restore to man his birthright of health and happiness. Presently, the citizens of our sanitary civilization have come to look to doctors, surgeons, their hospitals,

drugs, globulins, and vaccines for relief from the miseries of disease. Slowly, there is now emerging the sophisticated view that health is more than the absence of disease. It is a positive attribute, which permits man the fullest possible expression of his potentialities—physical and mental—for creative work and happiness. Health cannot be dissociated from the particular conditions under which man has to function. It implies fitness to his total environment. Because this view implies that so many independent and complex factors play a determinant role in the control of health, it does not lend itself readily to the formulation of practical programs of action. Instead of constituting a precise operational concept, it is for the time being no more than a somewhat vague philosophical attitude, but one which, nevertheless, must preside over all the campaigns directed against specific diseases.

History shows that tuberculosis is the product of incomplete civilization. It is a struggle in which civilized man is still at a biological disadvantage because imperfectly adapted to the new mode of life which he is creating or which is forced upon him. Tuberculosis is a social problem involving many factors other than the tubercle bacillus. It will be rapidly mastered only if society can be more perfectly fitted to human needs, limitations, and urges.

A FATAL CASE OF TUBERCULOSIS PRODUCED BY BCG[1]

JOHANNES MEYER

Microbiologic Studies by K. A. Jensen

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In January, 1943, a healthy boy was born in a baker's home in a small town in Western Zealand, Denmark. The parents were and still are in completely good health. They have had no other children.

Until 1950, when, at the age of seven, the boy was vaccinated with BCG, he had been in completely good health except for *German measles* in 1944 and *measles* in 1945. Both diseases were mild and without complications of any kind. In addition, he had *whooping cough* in 1949, of which further mention will be made.

The Holbæk County Tuberculosis Centre, which for many years has had a thorough knowledge of all patients with tuberculosis in the small town and its environs, has closely examined the possibility of contact between this patient and the few patients with open pulmonary tuberculosis in this district. No such contact could be demonstrated.

It may also be mentioned that the boy had never been given raw milk—only pasteurized milk from the dairy.

In November, 1949, the boy became ill with *whooping cough*. It was a very mild case, and the boy had made a complete recovery by Christmas, 1949. In February, 1950, he had a high fever one day, 102.2° to 104°F., without any known cause. The temperature fell to normal in the course of a few days after confinement to bed for eight days in all. He was then in completely good health, well and gay, until he was first sent to school on April 1, 1950.

Soon after the boy had been sent to school he had a tuberculin test (Moro) done on June 3, 1950. As this was entirely negative, he was vaccinated with BCG on June 7, 1950. On the same day, 29 other children in the school were inoculated with the same vaccine. None of these 29 children had any complications whatever in connection with the vaccination.

But the case of this patient was different.

A fortnight after the vaccination, he developed pain in the left shoulder and felt poorly. A month later, a node was noticed in the left axilla, and two months later an abscess had formed there, while at the same time there was a slight generalized swelling of the peripheral lymph nodes. After incision of the abscess by the patient's physician (who did not have the pus examined for tubercle bacilli), a fistula formed in the axilla and the patient felt persistently ill with periodic slight fever. The fistula closed in the course of the autumn, but the boy remained weak and tired, and from Christmas, 1950, he ran a constantly increased temperature. As his condition did not improve, he was admitted, in March, 1951, to the medical department of the Holbæk Central Hospital.

[1] From the Department of Surgical Tuberculosis, the Finsen Institute, Copenhagen, Denmark.

The examination at the Holbæk Hospital revealed tubercle bacilli in the stomach lavage, whereas a roentgenogram of the lungs showed nothing definitely abnormal. The patient was then transferred to the Tuberculosis Sanatorium for Children at Vintersbølle on April 18, 1951.

It is worth mentioning that a type determination was made of the tubercle bacilli cultivated from the patient's stomach lavage, and that Statens Seruminstitut, Copenhagen, replied that the tubercle bacilli were of *human type*. It was therefore considered that the patient's pulmonary tuberculosis could have no causal relationship to the BCG vaccination, but must be due to a superinfection.

During the patient's stay at the sanatorium at Vintersbølle, his temperature was very high, and gradually he developed large, multiple swellings of the lymph nodes, partly on the left side of the neck, partly preauricularly on both sides, in both groins, and in both elbow regions, the last with abscess formation. Roentgenograms of the lungs showed nothing abnormal in the beginning, but in the course of a few months there appeared an increase of width of the mediastinal shadow, which was interpreted as a congestive abscess around the spine. Tubercle bacilli were still present in the stomach lavage and were still stated to be of human type by Statens Seruminstitut.

Owing to the suspicion of an abscess around the spine and the abscess-forming adenitis, the patient was transferred, in August, 1951, to Kysthospitalet (seaside hospital) at Refsnæs.

On admission, the illness was characterized as a rather severe general infection with high fever and severe anemia (50 "per cent" hemoglobin). The only signs of localized tuberculosis were the multiple peripheral adenitis with abscess formation in both elbow regions and the previously mentioned increase of width of the mediastinal shadow. There were no signs of Pott's disease, and the increased mediastinal shadow was therefore considered indicative of the presence of an abscess originating from the mediastinal lymph nodes.

A tuberculin test (Mantoux) was only faintly positive (100 T.U.).

Soon after admission an incision was made in the abscess over the right cubital nodes. The pus was sent to Statens Seruminstitut, Copenhagen, and to Professor K. A. Jensen at the Institute of Pathology, University of Copenhagen, with the request that the pus be especially examined with a view to the possible presence of tubercle bacilli of the BCG type. Prolonged examinations in both laboratories then showed that, with regard to morphology of the colonies, virulence, et cetera, the tubercle bacilli found *could not be distinguished from organisms of the BCG type*. Professor K. A. Jensen further said that the case could hardly be one of mixed infection with virulent human tubercle bacilli, as not a single case of progressive tuberculosis had been observed among the numerous animal subjects inoculated with material or cultures derived from the patient.

The same result was arrived at in examinations of all other material sent in later, whether pus, gastric lavages, urine, or tissue specimens removed at autopsy.

After examination of the first two samples sent in from Holbæk and Vintersbølle, Statens Seruminstitut stated, as already mentioned, that the tubercle bacilli were of human type. This was simply due to the fact that the first samples were not examined especially with a view to the presence of BCG. The bacteriologic diagnosis of the BCG strain can only be established after especially prolonged cultivation on nutrient media and after virulence examinations in animals. When such examinations are not performed, the BCG type cannot be distinguished from the human type. As these special examinations were *not* performed in the first two samples, the strain was *wrongly stated to be of human type*.

The patient had been treated at Vintersbølle on a general sanatorium regimen and chemotherapy. From May 1 to June 18, 1951, the chemotherapy consisted of dihydrostreptomycin, 0.5 gm. twice daily (42 gm. total) and para-aminosalicylic acid (PAS), 1 gm. six times daily (294 gm. total). The chemotherapy was continued in this hospital, where, from August 18 to November 9, 1951, the patient was given dihydrostreptomycin, 0.5 gm. once daily (40.5 gm. total) and sodium PAS, 1 gm. four times daily (324 gm. total). In addition, he was given repeated blood transfusions, which caused the hemoglobin to rise to normal.

Otherwise the treatment was but of slight effect. The boy, who had not been very exhausted on admission, soon became worse, his appetite decreased, and his weight sank rapidly, while at the same time he developed enlargement of the spleen and the liver. An increasing number of the enlarged lymph nodes formed abscesses with fistula formation and large tuberculous ulcers preauricularly, on the neck and at the site of vaccination. Chemotherapy soon had to be abandoned owing to the anorexia.

As the patient's condition gradually threatened to become fatal, he was transferred, in November, 1951, to the Finsen Institute in Copenhagen, as it was considered that the possibilities of examination and treatment were better there.

But in spite of all attempts at parenteral nutrition and in spite of repeated blood transfusions, the patient's condition did not improve. Continued chemotherapy was attempted, but now met with great difficulties owing to the patient's exhausted condition. Treatment with PAS could not be carried through because of vomiting. From December 7, 1951, to January 30, 1952, the patient was therefore given dihydrostreptomycin, 0.5 gm. once daily (26 gm. total). The tubercle bacilli in pus which had previously been highly susceptible to streptomycin now became drug resistant. Subsequently, from March 22 to April 19, 1952, the patient was treated with isoniazid, 50 to 75 mg. daily (the patient's weight was 15.1 kg.), for a total of 1,850 mg. in all. But, as the patient then developed attacks of clonic convulsions of the extremities, it was not advisable to continue this treatment even though the relationship between the isoniazid and the convulsions was doubtful.

Soon after admission to Finsen Institute, before treatment with isoniazid, the patient's temperature became normal and subnormal, and the signs of enlargement of the liver and the spleen subsided, but the clinical picture was still marked by a very severe *chronic* intoxication.

The patient was extremely emaciated with almost complete wasting of the muscles, so that actually he presented the familiar clinical picture seen at the end of World War II in liberated prisoners in the German concentration camps.

Otherwise the picture was dominated by the multiple enlarged lymph nodes with abscess formation and surmounting tuberculous ulcerations and infiltrations of the skin, typical "scrofuloderma," chiefly localized to the face, the neck, and the left shoulder region. The roentgenographic signs of enlargement of the mediastinal nodes persisted, and it was considered that large conglomerations of lymph nodes could now be palpated in the abdomen.

Signs of tuberculosis of the viscera, or, perhaps more specifically, signs of hematogenous tuberculosis, were not yet found. The lung fields were clear; the urine had contained tubercle bacilli on one occasion, but was otherwise completely normal. No signs of tuberculosis of joints or bones were found.

The clinical picture was such a special one that the writer believes that relatively few present-day doctors have seen such a case, with widespread severe tuberculosis, localized exclusively to the lymphatic system. In descriptions by the ancient clinicians, however, one can read of a clinical picture which has now practically disappeared: *the severe malignant form of scrofulosis.* In so far as can be determined, the patient presented exactly this clinical picture.

The rest of the case record is as short as it is sad.

It was not possible in any way to obviate the severe intoxication or improve the general health.

The boy developed increasing edemas and also had attacks of clonic convulsions and unconsciousness (without signs of meningitis). Toward death the temperature rose again, and eventually roentgenographic signs of infiltrations in the lungs appeared shortly before death. After two years' disease the boy died at last in extreme cachexia on June 10, 1952.

Post-mortem examination was performed by Professor Engelbreth-Holm, The Institute of Pathological Anatomy, University of Copenhagen, and the present description is based exclusively on his autopsy report.

The examination showed a peculiar picture of what Professor Engelbreth-Holm termed an *atypical tuberculosis:*

> Both lungs were the seat of widespread, confluent, gritty, and dry infiltrates of rather a firm consistency. There were no signs of miliary tubercles, peribronchitis, or caseous necroses. In the left apex was an abscess of cherry-size.
>
> The most conspicuous change in the thorax was, however, an enormous enlargement of all lymph nodes. These enlarged nodes surrounded the trachea, occupied both hila and most of the mediastinum. Hardly any normal tissue was seen in sections through these lymph nodes. The tissue had been replaced by a necrotic mass of a peculiar rubber-sponge appearance and several decaying areas. Two perforations to the main bronchus were seen.
>
> The aorta and the vena cava were unchanged themselves but were surrounded in almost their entire course by enormous infiltrates, presumably originating from lymph nodes. They formed a "cloak" around the aorta, measuring 3 to 4 cm. in thickness and showed in sections the same picture as the bronchial lymph nodes. Several large abscesses were found retroperitoneally.

The entire mesentery was full of lymph nodes measuring up to 3 by 5 cm.

On the lesser curvature of the stomach, there were perforations into necrosing lymph nodes, just as in the main bronchus.

The *spleen* was slightly enlarged, without signs of tuberculosis.

The *liver* was of normal size, without signs of tuberculosis.

The *kidneys* showed on their surface numerous yellowish infiltrates, measuring approximately 1 by 1 cm., which, however, extended only a few millimeters into the renal tissue. Sections of the kidneys were otherwise completely normal.

Histologic examination of tissue of the large lymph nodes and the diffuse infiltrates surrounding the aorta showed a picture characterized by very widespread caseous necroses with abscess formation, but with slight tissue reaction with granuloma formation.

After staining for tubercle bacilli, the necrotic tissue proved to contain so many organisms that it was intensely red with low-power magnification.

Histologically, the gritty infiltrates in the lungs presented a very peculiar picture. In the infiltrates the alveolar walls were thickened, contained numerous cells, and, as the most essential change, diffuse amorphous calcium deposits in the connective tissue stroma. There were no tuberculous changes here, and, apart from the necrotic cavern in the left apex, tubercle bacilli could not be demonstrated in the lungs. Professor Engelbreth-Holm wrote that he could not categorize the findings and had no explanation to offer for the atypical pathologic changes.

Bacteriologic examination was made at the Institute of Pathology, University of Copenhagen, by Professor K. A. Jensen of specimens of mesenteric nodes, infiltrates from the abdomen, the spleen, kidneys, lungs, and liver. Tubercle bacilli were isolated by culture from all specimens and showed the same growth characteristics and virulence as the tubercle bacilli cultivated from pus, stomach lavage, and urine while the patient was alive. All cultures consisted of tubercle bacilli with the characteristics of typical BCG cultures.

Comment and summary: This case record speaks for itself and, therefore, only a few brief comments will be made. It is believed that the case record and the uniform result of the numerous bacteriologic examinations justify the following conclusions.

Bacillus Calmette-Guérin introduced by BCG vaccination *may* produce a fatal tuberculosis in man.

The clinical picture was very peculiar and special—a widespread malignant tuberculosis involving only the lymphatic system. To every observer, layman as well as professional, the causal relationship of the disease to the BCG vaccination seemed beyond all doubt. The bacteriologic proof just confirmed what appeared to be an obvious fact. The reason why not a single case of this nature has been reported among the millions of children who have been subjected to BCG vaccination must be that the case was *exceptionally rare*.

Consequently, this case *alone* should have no influence on current practice with respect to BCG vaccination.

MICROBIOLOGIC STUDIES BY DR. K. A. JENSEN OF TUBERCLE BACILLI ISOLATED FROM PATIENT DURING LIFE AND AT POST-MORTEM EXAMINATION

Professor K. A. Jensen, of the University Institute of Pathology, Copenhagen, has sent me the following report on the bacteriologic investigations that he has performed of pus and other material from the patient mentioned above.

Patient J.M.L.R., born January 19, 1943

I. Sample No. TB 6181: Pus from Fistula on the Upper Arm, Received October 22, 1951

Primary cultivation of Löwenstein medium with 0.75 per cent of glycerin yielded growth of approximately 50 eugonic colonies per test tube. The morphology of colonies was that of BCG or the human type. No growth in Besredka's medium.

Guinea pigs inoculated with the same pus sample were killed six weeks later and showed no signs of tuberculosis. Cultivation from the regional lymph node of the inoculated guinea pigs yielded growth of a few eugonic colonies resembling the human type or BCG.

Secondary cultivation: A fine suspension of the primary culture was prepared. Ten test tubes were inoculated with 10^{-5} mg. each and 10 tubes were inoculated with 10^{-6} mg. At the same time Besredka's medium was inoculated with 1 mg.

10^{-5} mg.: Ten times innumerable eugonic colonies resembling the human type or BCG.

10^{-6} mg.: In 10 tubes in all, 256 eugonic colonies resembling the human type or BCG. Besredka's medium: No growth.

Determination of virulence: This secondary culture was used for a determination of virulence. It is common to this and the following investigations that the various doses of tubercle bacilli were inoculated intraperitoneally into two guinea pigs and intravenously into one rabbit. The guinea pigs were killed two months, the rabbits three months, after inoculation.

Guinea pigs:

10^{-6} mg.: No tuberculosis.

10^{-4} mg.: No tuberculosis.

10^{-2} mg.: No tuberculosis.

1 mg.: In both animals, regressive tuberculous processes were seen in the omentum. The portal lymph node was somewhat enlarged, but not caseous. Spleen, liver, and lungs showed no tuberculous processes.

Rabbit:

10^{-2} mg.: No tuberculosis.

1 mg.: Lungs: A few, small regressive processes. Spleen, liver, and kidneys showed no tuberculous processes.

5 mg.: No tuberculosis.

Conclusion: The pure culture of the strain grew in the form of rather uniform, eugonic colonies on Löwenstein medium. The question whether it was a human

strain or BCG could not be settled from the morphology of the colonies. *According to previous and extensive experiences*, the fact that the strain did not grow in Besredka's medium, either primarily or secondarily, *definitely suggests that it was BCG.*

The virulence test showed that the strain had the same virulence as BCG. The local processes found in the guinea pigs inoculated intraperitoneally with up to 1 mg. of culture in no way exceeded those following inoculation of the same doses of BCG.

The determination of the virulence in guinea pigs and rabbits also shows that the virulence of the BCG culture had not increased while it was harbored by the patient. The investigation also shows that it was not a mixed infection of BCG and a human type. The virulence determination was performed with a culture cultivated from the greatest possible number of the primary colonies. In addition, the guinea pig inoculated with pus showed no macroscopic signs of tuberculosis.

The other pure cultures of strains obtained from the patient were examined in the same manner. As the findings with regard to growth and virulence were the same in all strains as in TB 6181, only a brief description will be given in the following.

II. Sample No. TB 6398. Necrotic Tissue from Left Preauricular Lymph Node Abscess, Received on November 13, 1951

Microscopic examination: Numerous tubercle bacilli.

Cultivation: Growth of innumerable eugonic colonies resembling the human type or BCG.

Besredka's medium inoculated primarily from the material or secondarily with the pure culture: No growth.

Guinea pigs, inoculated with the same material and killed six weeks later presented a local process of small extent and no other signs of tuberculosis.

From the local process and the regional lymph node, 8 eugonic colonies resembling the human type or BCG were cultivated.

Determination of virulence of the primary culture:

Guinea pigs:

10^{-6} mg.: No tuberculosis.

10^{-4} mg.: No tuberculosis.

10^{-2} mg.: No tuberculosis.

1 mg.: Omentum: A few small, regressive, tuberculous nodules, one with a central abscess, measuring approximately 1 mm. The portal lymph node was slightly enlarged, but not caseous. Spleen, liver, and lungs: no tuberculous processes.

5 mg.: Omentum: A few small, regressive, tuberculous nodules. The portal lymph node was somewhat enlarged, but not caseous. Lungs, liver, and spleen: no tuberculous processes.

Rabbit:

10^{-2} mg.: No tuberculosis.

1 mg.: No tuberculosis.

5 mg.: No tuberculosis.

III. Sample No. TB 6399. Pus from Left Preauricular Lymph Node Abscess, Received on November 13, 1951

Microscopic examination: Numerous tubercle bacilli.

Cultivation: Growth of numerous eugonic colonies resembling the human type or BCG.

Besredka's medium inoculated primarily with the material or secondarily with the pure culture: No growth.

Guinea pigs inoculated with the same material and killed six weeks later presented a local abscess of pea size, but otherwise no signs of tuberculosis. Cultivation from the abscess yielded a growth of a number of eugonic colonies resembling the human type or BCG.

Determination of virulence of the primary culture:

Guinea pigs:

10^{-6} mg.: No tuberculosis.

10^{-4} mg.: No tuberculosis.

10^{-2} mg.: No tuberculosis.

1 mg.: Omentum: A number of abscesses of a regressive nature; they were up to pea size. The portal lymph node was somewhat enlarged, but not caseous. Spleen, liver, and lungs were normal.

5 mg.: Omentum: This was transformed into a sausage-shaped mass of chronic tuberculous granulation tissue containing a few abscesses up to pea size. The processes were of a regressive nature. The portal lymph node was somewhat enlarged, but not caseous. Spleen, liver, and lungs were normal.

Rabbit:

10^{-2} mg.: No tuberculosis.

1 mg.: The animal died of a different cause. No signs of tuberculosis.

5 mg.: One kidney contained a tuberculous nodule the size of a millet seed; no other signs of tuberculosis.

IV. Sample No. TB 6926. Pus from Abscess, Received on January 22, 1952

Cultivation: Growth of a number of eugonic colonies resembling the human type or BCG.

Besredka's medium: No growth.

Guinea pigs inoculated with the same pus and killed six weeks later presented a local process about the size of a millet seed; no other signs of tuberculosis.

Determination of virulence of the primary culture:

Guinea pigs:

10^{-2} mg.: No tuberculosis.

1 mg.: Omentum: Abscesses of a regressive nature, up to pea size. The portal lymph node was somewhat enlarged, but not caseous. Spleen, liver, and lungs were normal.

5 mg.: The omentum was transformed into a sausage-shaped mass of tuberculous granulation tissue of a regressive nature. A few small abscesses were present. The portal lymph node was enlarged, not caseous. Spleen, liver, and lungs were normal.

Rabbit:

1 mg.: No tuberculosis.

5 mg.: No tuberculosis.

Samples TB 7060 and 7435: Urines Received on February 6 and March 21, 1952

Cultivation: Culture sterile.
Inoculation into guinea pigs: No "takes" of tuberculosis.

V. Sample No. TB 7443. Pus from Abscess on the Neck, Received on March 22, 1952

Microscopic examination: Numerous tubercle bacilli.
Cultivation: Growth of innumerable eugonic colonies resembling the human type or BCG.
Besredka's medium: No growth.
Guinea pigs inoculated with the same pus and killed six weeks later: An abscess of pea size appeared at the site of inoculation; no other signs of tuberculosis.

Determination of virulence:

Guinea pigs:
10^{-3} mg.: No tuberculosis.
1 mg.: Omentum: A few small abscesses; no other signs of tuberculosis.
5 mg.: Omentum: A few small abscesses; no other signs of tuberculosis.
Rabbit:
1 mg.: No signs of tuberculosis.

EXAMINATION OF AUTOPSY MATERIAL RECEIVED ON JUNE 11, 1952

VI. TB 8564. Mesenteric Lymph Node

Cultivation: Growth of innumerable eugonic colonies resembling the human type or BCG.
Besredka's medium: No growth.

Determination of virulence:

Guinea pigs:
10^{-3} mg.: Omentum: A few noncaseous nodules.
1 mg.: No tuberculosis.
5 mg.: Omentum: Three nodes the size of millet seeds.
Rabbit:
5 mg.: No tuberculosis.

VII. TB 8565. Retroperitoneal Infiltrate

Cultivation: Growth of innumerable eugonic colonies resembling the human type or BCG.
Besredka's medium: No growth.

Determination of virulence:

Guinea pigs:
10^{-3} mg.: No tuberculosis.
1 mg.: No tuberculosis.
5 mg.: No tuberculosis.
Rabbit:
5 mg.: No tuberculosis.

VIII. TB 8566. Spleen

Cultivation: Growth of a number of eugonic colonies resembling the human type or BCG.
Besredka's medium: No growth.

Determination of virulence was not performed.

IX. TB 8567. Left Kidney

Cultivation: Growth of a number of eugonic colonies resembling the human type or BCG.
Besredka's medium: No growth.

Determination of virulence:

Guinea pigs:
10^{-2} mg.: No tuberculosis.
1 mg.: Omentum: A number of nodes up to pea size, some with purulent contents. Spleen, liver, and lungs were normal.
5 mg.: The omentum was transformed into a thick, sausage-shaped mass of granulation tissue with abscesses of pea size. The portal lymph node was enlarged, but without caseous contents.
Rabbit:
5 mg.: No tuberculosis.

X. TB 8568. Processes from the Right Lungs

Cultivation: Growth of a number of eugonic colonies resembling the human type or BCG.
Besredka's medium: No growth.

Determination of virulence:

Guinea pigs:
10^{-2} mg.: No tuberculosis.
1 mg.: Omentum: A number of abscesses up to pea size; no other signs of tuberculosis.
5 mg.: Both guinea pigs died of different causes three to four weeks after inoculation. Except for local processes in the omentum, there were no signs of tuberculosis.
Rabbit:
5 mg.: One kidney contained a few nodes up to the size of millet seeds; there were no other signs of tuberculosis.

XI, XII, XIII. TB 8569 (Liver), TB 8562 (Pus from Axilla), and TB 8563 (Ascites Fluid)

Cultivation: All three samples produced a growth of a number of eugonic colonies resembling the human type or BCG.
Besredka's medium: No growth.

Determination of virulence was not performed.

Conclusion: Thirteen tubercle bacilli in all showing uniform conditions of growth and virulence have been obtained in pure cultures from the patient J. M. L. R. at varying times and from different localizations.

They all grew in eugonic colonies resembling the human type or BCG. None of the strains grew in Besredka's culture medium; this is characteristic of BCG. The virulence in guinea pigs and rabbits was determined with 9 of the 13 strains.

The 9 strains showed the same virulence as BCG; it must therefore be concluded that the strains of tubercle bacilli found were BCG. The investigation also showed that the infection had been caused by BCG *only*. In spite of the innumerable inoculations into guinea pigs of the material from the patient and of the numerous cultures obtained from the patient, it was not possible to demonstrate the presence of a single virulent tubercle bacillus.

Consequently, there can have been no superinfection with virulent tubercle bacilli.

It may be mentioned that experience of many years has shown that, in patients infected with tubercle bacilli of the human or the bovine type, tubercle bacilli of the attenuated virulence described above will never develop owing to chemotherapy with streptomycin and PAS. Moreover, in the present case, the first five samples for bacteriologic examination were obtained before the patient was treated with isoniazid.

THE CHEMOTHERAPY OF PULMONARY TUBERCULOSIS WITH PYRAZINAMIDE USED ALONE AND IN COMBINATION WITH STREPTOMYCIN, PARA-AMINOSALICYLIC ACID, OR ISONIAZID[1, 2]

WILLIAM S. SCHWARTZ AND RALPH E. MOYER

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INTRODUCTION

Following the synthesis of pyrazinamide during a survey of compounds related to the thiosemicarbazones and nicotinamide by Kushner and associates (1), it was established by Malone (2), by Dessau (3*a*), and by Solotorovsky (3*b*) and their respective associates that the compound possessed some degree of effectiveness against tuberculosis in experimental animals. Successful demonstration of the antituberculous action of pyrazinamide in man was made by Yeager, Monroe, and Dessau (4, 5), and a clinical study of the compound was begun at this hospital in September, 1951. Up to the present time, a total of 181 patients have received 202 courses of treatment with pyrazinamide for periods up to sixteen months (6, 7, 8*a*), either alone or in combination with streptomycin, para-aminosalicylic acid (PAS), or isoniazid. As it had been learned early in the study of this drug that its effect, although definite when used alone, was relatively short (4, 5, 6), it was used in multiple-drug therapy. This was done with the hope that the total drug action would be prolonged and the over-all clinical value increased over that which would have been the case if one of the drugs used in the pairings had been used alone. Failure in this hope occurred from the pairing of pyrazinamide and PAS and of pyrazinamide and streptomycin, but the pairing of pyrazinamide and isoniazid proved to be successful.

PLAN OF INVESTIGATION

Selection of patients: The great majority of the patients treated on any of the regimens were those who had previously been unsuccessfully treated with streptomycin-PAS, as well as with bed rest and collapse or surgical therapy, whenever the last-named were feasible. A few patients who had become hypersensitive to streptomycin or PAS were also included. Moreover, with the regimens containing streptomycin or PAS, a few patients with far advanced cavitary disease who had not had previous chemotherapy were treated with the hope of expediting the study of the incidence of drug-resistant organisms. Among the patients who received pyrazinamide alone, a particular attempt was made to select patients with fever and pulmonary lesions with a recent progressive component added to long-standing chronic disease in order to assess therapeutic efficacy. As the study of the multiple-drug regimens was originally planned to

[1] From the Veterans Administration Hospital, Oteen, North Carolina.

[2] Portions of this study were presented at the Veterans Administration-Army-Navy Conferences on Chemotherapy held at Atlanta, Georgia, February 9–12, 1953, and at St. Louis, Missouri, on February 8–11, 1954.

be chiefly one of bacterial resistance and toxicity, patients with recent acute spreads treated with any of these regimens were not common. As may be deduced from the foregoing, the type of pulmonary lesions studied would not have been expected to do particularly well under any treatment regimen.

Regimen: As reported in the tables, pyrazinamide alone, 2.8 gm. daily, was given for only 42 days in most cases, for it had been determined that evidence of loss of clinical effectiveness was likely to occur after one or two months of treatment (4, 5). In a few cases, treatment was continued for 84 days. Pyrazinamide, 3 gm. daily, and streptomycin, 1 gm. twice weekly, were given for four to twelve months. Pyrazinamide, 3 gm. daily, and PAS, 12 gm. daily, were given for four to twelve months. Pyrazinamide, 3 gm. daily, and isoniazid, 300 mg. daily, were given for four to sixteen months.

In all, pyrazinamide alone was given to 19 patients; pyrazinamide and PAS to 53 patients; pyrazinamide and streptomycin to 28 patients; and pyrazinamide and isoniazid to 102 patients. Discrepancies in these totals with the numbers shown in the tables are due to exclusion of patients who had received one of the drugs previously or, in some circumstances, when the duration of treatment had been too short. No cases, however, were excluded from review with respect to toxicity.

Laboratory studies: The patients who received pyrazinamide alone had examinations of the urine, blood urea nitrogen, complete blood count, prothrombin time, bromsulfalein test, cephalin flocculation, icteric index, and electrocardiogram before and weekly during treatment. Roentgenograms of the chest were obtained every two weeks, and weekly attempts were made to determine the presence of pyrazinamide-resistant tubercle bacilli in the sputum of those discharging bacilli. When the regimens of pyrazinamide and another drug were studied, examinations of the urine and blood were made once a month during treatment, and electrocardiograms were omitted. Chest roentgenograms were at first taken monthly and later every two months. Drug-susceptibility studies of tubercle bacilli isolated from the sputum were made at two-week intervals and later at monthly intervals for all drugs involved. Attempts to determine the susceptibility of tubercle bacilli to pyrazinamide were discontinued on April 14, 1953. Because of the lack of evidence of liver toxicity of pyrazinamide at that time, routine liver function tests were also discontinued; these were resumed in February, 1954.

The pyrazinamide concentrations were determined by a method developed in Lederle Laboratories and furnished to the present writers by Dr. James Ruegsegger (86).

RESULTS

Pyrazinamide Alone

Treatment of the first patient in the pyrazinamide alone series was begun in September, 1951, and a total of 19 patients were treated on the regimen. The results are presented in table 1. While the results did not seem impressive, it may be remembered that the proportion undergoing moderate, or marked, roentgenographic improvement was quite similar to that of a series of patients treated with streptomycin alone for 42 days (9).

In terms of roentgenographic improvement at six weeks, 9 showed marked or moderate improvement, 2 were worse, and 10 were only slightly improved or unchanged. Four of the group were treated for an additional six weeks, and 2

TABLE 1

PYRAZINAMIDE ALONE (2.8 GM. DAILY) IN 19 PATIENTS WITH PULMONARY TUBERCULOSIS

DURATION	42 DAYS	84 DAYS
Roentgenogram, marked or moderate improvement	7	2
Roentgenogram, slight improvement or no change	10	1
Roentgenogram, worse	2	1
Sputum positive for tubercle bacilli	16	4
Sputum negative for tubercle bacilli	3	0
Treatment stopped—end of course	15	3
Treatment stopped—toxicity	1	0

of the 4 showed marked or moderate improvement at that time. Tubercle bacilli could be isolated from 16 of the 19 patients after six weeks of treatment with pyrazinamide alone.

In comparing these data for pyrazinamide alone with those obtained from various pairings of pyrazinamide with other drugs, it should be remembered that a larger proportion of the patients in the pyrazinamide alone group had roentgenographic evidence of recent and, hence, more reversible components of disease than did the patients on the multiple drug regimens. All patients who received pyrazinamide alone promptly experienced a marked improvement in feeling of well-being. Cough and expectoration diminished. Fever was abolished in three or four days in 6 patients who were febrile before treatment. However, in one of these patients, daily fever as high as 100°F. returned shortly before the end of treatment and persisted subsequently. This was thought to be due to loss of suppressive action of the drug rather than to drug toxicity. Appetite improved, and all but one who remained stationary gained in weight. The gain varied from 4 to 15 pounds, the average being 9 pounds. One patient who had actively progressing far advanced pulmonary tuberculosis, as well as severe tuberculous ulceration of the pharynx and soft palate, is worthy of special comment. The ulcers had developed during treatment with streptomycin and PAS. Tubercle bacilli cultured from the sputum were known to be highly resistant to streptomycin. Treatment with penicillin and later with chlortetracycline had no visible or symptomatic effect on the ulceration. Within two days of the beginning of pyrazinamide therapy, the patient was able to eat a regular diet, whereas before he was able to swallow only liquids with great difficulty. The lesions receded considerably within a week and disappeared within two weeks.

The concentrations of pyrazinamide in the blood were determined in 2 patients who were receiving 2.8 gm. daily. On the fifteenth day of treatment, the pyrazinamide concentrations were 57 and 46 mg. per ml. at 8 a.m., 92 and 85 at 10 a.m., and 103 and 96 mg. per ml. at 10 p.m., respectively.

Attempts to determine *in vitro* the pyrazinamide susceptibility or resistance of tubercle bacilli derived from the patients were unsuccessful. Pretreatment inhibition of growth of tubercle bacilli occurred with as few as 25 γ per ml. or failed to occur in the presence of 1,000 γ of pyrazinamide per ml. There was

TABLE 2

PYRAZINAMIDE-PAS IN 35 PATIENTS WITH PULMONARY TUBERCULOSIS

(Regimen: Pyrazinamide, 3 Gm. Daily; PAS, 12 Gm. Daily)

DURATION (MONTHS)	<2	2	4	6	8	10
Roentgenogram, marked or moderate improvement		6	8	4	2	1
Roentgenogram, slight improvement or no change		18	5	0	0	0
Roentgenogram, worse		5	8	0	0	0
Sputum positive for tubercle bacilli		21	13	0	1	0
Sputum negative for tubercle bacilli		8	7	4	1	1
Treatment stopped—worse	1	6	7	0	0	0
Treatment stopped—left against medical advice	2	1	4	0	0	0
Treatment stopped—toxicity	2	0	1	0	0	0
Treatment stopped—resistant to pyrazinamide	1	3	1	1	0	0

also complete lack of correlation of *in vitro* drug-susceptibility tests and the clinical course of the patients while under treatment. A general impression, arrived at solely on clinical grounds, was formed, however, to the effect that, when used alone, pyrazinamide lost its effectiveness in most cases after one or two months of therapy. This led to the conclusion that pyrazinamide alone was chiefly useful in tiding patients over an acute episode or in providing chemotherapeutic coverage for a short time during excisional thoracic surgical procedures. Its usefulness with respect to the latter has already been shown (10).

Pyrazinamide and Para-Aminosalicylic Acid

Thirty-five patients were started on treatment with pyrazinamide-PAS, with 23 completing two months and 21 completing four months of therapy on this regimen. Only 4 patients were maintained on the pyrazinamide-PAS regimen for as long as six months (table 2). Treatment was discontinued in 6 patients because of the concept then held, but later found to be erroneous, that *in vitro* pyrazinamide-resistance tests were meaningful.

Unfortunately for the evaluation of the influence of PAS, all but one patient had received it previously. The tubercle bacilli derived from 16 patients were known to be PAS-susceptible and the bacilli from 12 patients were known to be PAS-resistant before treatment with pyrazinamide-PAS was started. Of the 16 patients who discharged PAS-susceptible strains, 4 developed PAS-resistant strains during pyrazinamide-PAS therapy, and the remainder stopped discharging tubercle bacilli in the sputum. Of those with PAS-resistant strains before pyrazinamide-PAS treatment, 7 continued to discharge such PAS-resistant strains and the remainder became noninfectious. The tentative conclusions which could be drawn seemed to agree with those reached by clinical means, namely, that pyrazinamide-PAS was an unsatisfactory regimen for long-term chemotherapy in that PAS did not prolong the effectiveness of pyrazinamide.

Pyrazinamide and Streptomycin

A total of 16 patients were started on treatment with pyrazinamide-streptomycin. As with pyrazinamide-PAS, the pyrazinamide-streptomycin regimen

TABLE 3

PYRAZINAMIDE-STREPTOMYCIN IN 16 PATIENTS WITH PULMONARY TUBERCULOSIS
(Regimen: Pyrazinamide, 3 Gm. Daily; Streptomycin, 1 Gm. Twice Weekly)

DURATION (MONTHS)	2	4	6	8	10
Roentgenogram, marked or moderate improvement	1	2	1	0	0
Roentgenogram, slight improvement or no change	14	9	2	2	1
Roentgenogram, worse	1	1	0	0	0
Sputum positive for tubercle bacilli	12	9	2	2	0
Sputum negative for tubercle bacilli	4	2	1	0	1

proved unsatisfactory and only a few patients were maintained on this regimen for longer than four months (table 3). Moderate or marked roentgenographic improvement seldom occurred; a relapse occurred in one patient. The incidence of "sputum conversion" was unimpressive.

Evidence of the delay of the emergence of streptomycin-resistant tubercle bacilli was likewise lacking. Only 3 of the 16 patients had received no previous streptomycin, but all 3 were discharging streptomycin-resistant tubercle bacilli by the fourth month of pyrazinamide-streptomycin therapy. Of the 7 patients previously treated with streptomycin, but still with drug-susceptible strains, 6 stopped discharging tubercle bacilli but the other developed a streptomycin-resistant strain. Of 4 patients with streptomycin-resistant strains before treatment, one had cultures negative for tubercle bacilli and 3 showed the continued presence of streptomycin-resistant organisms. Thus, there was no evidence that pyrazinamide along with streptomycin prevented or delayed the emergence of streptomycin-resistant tubercle bacilli.

Pyrazinamide and Isoniazid

Thirty patients with pulmonary tuberculosis were started on the pyrazinamide-isoniazid regimen. All of these 30 had received previous chemotherapy which did not include either pyrazinamide or isoniazid, and all were discharging streptomycin-resistant strains of tubercle bacilli when pyrazinamide-isoniazid therapy was started. Isoniazid and pyrazinamide had been used rather extensively at this hospital, either singly or in combination with other drugs. It is for this reason that there were so relatively few, i. e., 30, patients with pulmonary lesions suitable for drug evaluation who had not received either drug previously. Moreover, all of these 30 patients not only had streptomycin-resistant strains of tubercle bacilli but represented clean-cut failures from the use of other regimens of chemotherapy.

With due allowance for the very unfavorable type of case selected, it is believed that the results observed were excellent (table 4).

Moderate or marked roentgenographic improvement was observed in 15 of 29 patients (52 per cent) *at four months*; in 15 of 19 patients (79 per cent) *at eight months*; and in 6 of 7 patients (86 per cent) *at twelve months*. In only one patient was there roentgenographic evidence of progression of disease. Cavity closure had occurred at four months in 8 of the 28 cavitary cases (29 per cent). At eight

TABLE 4

PYRAZINAMIDE-ISONIAZID IN 30 PATIENTS* WITH PULMONARY TUBERCULOSIS
(Regimen: Pyrazinamide, 3 Gm. Daily; Isoniazid, 300 Mg. Daily)

TOTAL CASES	ROENTGENOGRAPHIC CHANGE†			CAVITY CHANGE		SPUTUM
	M-M	S-O	W	Number with Cavity	Cavity Closed	Negative Culture
4 months 29	15 (52%)	14	0	28	8 (29%)	24 (83%)
8 months 19	15 (79%)	3	1	18	10 (56%)	19 (100%)
12 months 7	6 (88%)	1	0	7	2 (43%)	7 (100%)

* One patient had a pneumonectomy before completion of four months' therapy and has been excluded from the analysis.

† M-M. Moderate or marked roentgenographic improvement
S-O. Slight roentgenographic improvement or no change
W. Roentgenographic evidence of relapse

months, cavity closure had occurred in 10 of the 18 patients (56 per cent) with cavity observed for that period, and at twelve months in 2 of the 7 patients (43 per cent) treated for that period.

Tubercle bacilli could be cultured from only 5 of 29 patients after four months of pyrazinamide-isoniazid therapy. No tubercle bacilli could be cultured thereafter from the 19 patients studied at eight months or from the 7 studied at twelve months. Attainment of sputum cultures negative for tubercle bacilli in 83 per cent (24 of 29 patients) at four months and in all cases subsequently, in spite of the fact that cavities persisted in a considerable proportion of patients, indicated a high degree of continuing antibacterial action.

It appeared that the effectiveness of the pyrazinamide-isoniazid regimen compared favorably with that of the regimens of streptomycin, PAS, and isoniazid presently in use in various combinations and in generally more favorable cases. Moreover, the present results with pyrazinamide-isoniazid were superior to those attained in a similar group of 88 patients treated for approximately four months with isoniazid alone (11). In the latter series, only 12 per cent showed moderate or marked roentgenographic improvement and 25 per cent "conversion" of sputum by the fourth month, while 22 per cent of the patients experienced a relapse during that time.

With the intent of determining whether or not pyrazinamide would delay the emergence of isoniazid-resistant organisms, the patients chosen represented in a large proportion those who would be expected to continue to discharge tubercle bacilli. Unfortunately for the study, but fortunately for the patients, the high proportion of sputum cultures negative for tubercle bacilli made the collection of significant data impossible. Only 5 patients were discharging tubercle bacilli in the sputum at the fourth month of pyrazinamide-isoniazid treatment. Three of these patients' strains were susceptible to less than 0.2 γ of isoniazid per ml., and two of the strains were resistant to 5 γ per ml. For the sake of completeness, it is reported that one patient not noted in the table had a pneumonectomy performed successfully during the first four months of treatment.

Treatment was continued for a total of eight months, and he has continued to do well. Two of the patients received thoracoplasty after eight months of pyrazinamide-isoniazid treatment, while chemotherapy was continued for twelve months. The results were satisfactory, but the patients were dropped from the tabulation at eight months.

Six patients received pneumoperitoneum prior to and during chemotherapy; in one other, it was added at the beginning of chemotherapy. All cavities disappeared in 4 of these 7 cases.

The combination of pyrazinamide and isoniazid was also used in a number of patients who had received one or the other of the drugs previously. A satisfactory result was not attained in most cases. Usually there was evidence of clinical or *in vitro* drug resistance to one or both drugs prior to the beginning of the use of pyrazinamide and isoniazid together. When improvement occurred, it was usually only temporary and was in accord with what might have been expected from the single use of the particular drug to which the patient's tubercle bacilli were susceptible.

Toxicity

Yeager and his associates (4), using pyrazinamide alone, reported the complaint of joint pains by 11 of 43 patients treated. In addition, in the same group, 2 patients developed clinical jaundice and 3 had drug fever. A moderate degree of gastrointestinal disturbance was also reported. They stated, however, that in a dosage of 2.8 gm. of pyrazinamide daily significant toxicity did not occur. Recently, McDermott and his associates (12), while reporting excellent clinical results from the use of 50 mg. of pyrazinamide per kg. and 5 mg. of isoniazid per kg. in 61 cases, found that clinical jaundice, presumably attributable to drug therapy, had occurred in 4 patients, in one of whom the hepatitis was fatal. Moreover, evidence of hepatitis without jaundice was observed in 2 additional patients. Campagna (13) reported mild jaundice in 3 of 21 patients who received pyrazinamide-isoniazid; the jaundice disappeared without interruption of treatment. A single case of jaundice during the use of pyrazinamide alone has been reported by Phillips and his associates (14).

The present investigation has been attended with good fortune in regard to toxicity. A few patients complained of joint pains, but in all instances it was found that these patients had suffered from arthritis and had had similar pains before therapy. No change was seen in serial electrocardiograms in these patients, and the joint pains did not increase in severity while therapy was continued. In one patient who received pyrazinamide alone, the drug was discontinued because of a slightly elevated retention of bromsulfalein, which promptly returned to normal without other findings. Of those whose pyrazinamide-PAS treatment was discontinued due to toxicity, it was stopped because of PAS intolerance in all but one patient who had a reduction in hemoglobin from 75 to 69 "per cent."

After two and one-half years of experience with 181 patients treated with pyrazinamide, either alone or together with PAS, streptomycin, or isoniazid, it has been necessary for the first time to discontinue pyrazinamide-isoniazid in

2 patients because of mild jaundice during the past month. The 2 patients had been receiving pyrazinamide in an approximate daily dosage of 42.4 and 44.8 gm. per kg. of body weight, respectively. The jaundice appeared after 72 and 149 days of pyrazinamide-isoniazid therapy.

Prior to this episode, treatment had been stopped in 3 patients because of suspected toxicity while receiving pyrazinamide-isoniazid. In one patient, the chemotherapy had been stopped because of an increase of abnormal values for the cephalin flocculation test, which had yielded abnormal values before treatment. In another patient, drug treatment was discontinued because of chills and fever, which had also occurred previously while he had been receiving streptomycin-PAS. In the third patient, the drug regimen was stopped because of extreme nervousness.

SUMMARY

Pyrazinamide used alone in a dosage of 2.8 gm. daily was found to be an effective drug in the treatment of pulmonary tuberculosis, although for only a short period of time.

The administration of streptomycin, 1 gm. twice weekly, or para-aminosalicylic acid (PAS), 12 gm. daily, together with 3 gm. of pyrazinamide daily did not enhance the effectiveness of pyrazinamide or streptomycin or delay the emergence of PAS-resistant or streptomycin-resistant tubercle bacilli.

It was not possible to determine the drug susceptibility of tubercle bacilli to pyrazinamide *in vitro*.

Chemotherapy with pyrazinamide and isoniazid has been found to be a very effective regimen in the treatment of pulmonary tuberculosis and to be at least the equal of any combination of streptomycin, PAS, or isoniazid in use at present. In spite of the reported toxicity, the low incidence of jaundice encountered in this series prompts the recommendation that the combination of pyrazinamide and isoniazid be used more widely in the treatment of tuberculosis, particularly in streptomycin-resistant cases.

SUMARIO

La Quimioterapia de La Tuberculosis Pulmonar con la Piracinamida Usada Sola y en Combinación con la Estreptomicina, el Acido Para-Aminosalicílico o la Isoniacida

La piracinamida, usada por sí sola a dosis de 2.8 gms. diarios, resultó ser una droga eficaz en el tratamiento de la tuberculosis pulmonar, aunque sólo por un breve período de tiempo.

La administración de estreptomicina, 1 gm. dos veces semanales, o de ácido para-aminosalicílico (PAS), 12 gms. diarios, junto con 3 gms. diarios de piracinamida, no acrecentó la eficacia de la piracinamida ni demoró la aparición de bacilos tuberculosos PAS-resistentes o estreptomicino-resistentes.

No fué posible determinar la farmacosusceptibilidad de los bacilos tuberculosos a la piracinamida *in vitro*.

La quimioterapia con piracinamida e isoniacida ha resultado ser un régimen muy eficaz en el tratamiento de la tuberculosis pulmonar y a lo menos igual a cualquier combinación de estreptomicina, PAS o isoniacida usada actualmente. A pesar de la supuesta toxicidad, la baja incidencia de ictericia observada en esta serie motiva la recomendación de que se use más ampliamente la combinación de piracinamida e isoniacida en el tratamiento de la tuberculosis, en particular en los casos estreptomicino-resistentes.

RESUME

Chimiothérapie de la tuberculose pulmonaire par la Pyrazinamide utilisée seule ou associée à la streptomycine, à l'acide para-aminosalicylique ou à l'isoniazide

La pyrazinamide utilisée seule à la dose de 2,8 g par jour a été trouvée efficace dans le traitement de la tuberculose pulmonaire, mais seulement pendant une période brève.

L'administration de streptomycine à la dose de 1 g deux fois par semaine, ou de P.A.S., 12 g par jour, en association avec 3 g de pyrazinamide par jour, n'a ni renforcé l'efficacité de la pyrazinamide ou de la streptomycine, ni retardé l'apparition de bacilles tuberculeux P.A.S. ou streptomycine-résistants. La sensibilité des bacilles tuberculeux à la pyrazinamide n'a pu être déterminée *in vitro*.

La chimiothérapie par la pyrazinamide et l'isoniazide a été trouvée un traitement très efficace de la tuberculose pulmonaire et considérée au moins égale à toute thérapeutique par l'association streptomycine, P.A.S. ou isoniazide actuellement en usage. En dépit de rapports sur la toxicité de ce composé, la faible incidence des cas d'ictère relevés dans cette série incite à recommander un usage plus étendu du traitement combiné pyrazinamide-isoniazide dans la tuberculose, en particulier dans les cas streptomycine-résistants.

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TOXICITY OF PYRAZINAMIDE[1]

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INTRODUCTION

Following a report of the antituberculous activity of nicotinamide in guinea pigs and mice (1, 2), Solotorovsky and associates (3), and Kushner and associates (4), independently, showed that pyrazinamide, an analogue of nicotinamide, was more active than nicotinamide in mice. Clinical trials were first reported by Yeager, Monroe, and Dessau (5) in 1952. Following Yeager's report, Schwartz and Moyer[2] (6, 7) reported that the combination of pyrazinamide and isoniazid gave unexpectedly good clinical response, as compared with the effect of either drug alone. The enhanced activity of this combination was likewise observed by McDermott and associates (8) and by Campagna, Calix, and Hauser (9). Two lines of experimental studies support the observation of high activity in man. McCune and Tompsett (8, 10) have shown that treatment of tuberculous mice with combinations of pyrazinamide and isoniazid lead to complete sterilization of lungs and spleen. The sterilizing effect could not be achieved with any other single agent or combination of agents. Solotorovsky and Ironson (11) have reported that pyrazinamide significantly retards the emergence *in vitro* of isoniazid-resistant tubercle bacilli.

A number of the investigators (5, 8, 9, 12) who have used pyrazinamide in man have reported the occurrence of hepatic toxicity. In view of the potential importance of pyrazinamide in the treatment of tuberculosis in man, it appears advisable to present the following studies on the toxicity of pyrazinamide in experimental animals.

MATERIALS AND METHODS

All animals were housed in air-conditioned quarters. An adequate stock diet and water were given *ad libitum*. Pyrazinamide was administered by stomach tube as a fine suspension in 10 per cent gum acacia.

Toxicity: The acute oral toxicity was determined in male white mice (IS32 strain), weighing between 18 and 20 gm. each, and male rats (Carworth strain), weighing between 100 and 120 gm. each. The effects of repeated daily administration of pyrazinamide were studied in young growing rats and in 6 adult dogs. The drug was given to six groups of 8 rats each for ninety days and to 6 dogs during a twenty-one to ninety-day period. To facilitate absorption and simulate clinical conditions, one half of the total daily dose was given in the early morning and the other half in the late afternoon. Details of the amount of drug given are presented in the section dealing with results.

Clinical laboratory tests: Aside from the daily observations of the physical condition and behavior, the following examinations were made at regular intervals throughout the study: body weight; food consumption; hematologic studies, including erythrocyte, leukocyte,

[1] From the Merck Institute for Therapeutic Research, Rahway, New Jersey; and Kings County Hospital, Brooklyn, New York.

[2] Reported in this number of the REVIEW (p. 413).

TABLE 1

ACUTE ORAL TOXICITY OF PYRAZINAMIDE IN MICE AND RATS

DOSE	MORTALITY*		REMARKS
	Mice	Rats	
gm. per kg.	*per cent*		
0.5	0	0	
1.0	0	0	
1.5	0		
2.0	0	0	Restlessness
2.5	0		Restlessness
3.0	30	30	Ataxia and depression
3.5	40		Ataxia and depression
4.0	100	30	Ataxia and depression

* 20 animals per group; ten-day observation period.

and differential leukocyte counts, hematocrit, hemoglobin, and erythrocyte sedimentation rate. Biochemical tests of the blood included urea nitrogen, total proteins, albumin, globulin, A/G ratio, inorganic phosphorus, alkaline phosphatase, cholesterol, cholesterol ester, fibrinogen, blood sugar, sodium, and carbon-dioxide combining power. The methods employed have been summarized elsewhere (13). Bromsulfalein tests for liver function were done when indicated. Urinalysis included color, pH, specific gravity, albumin, reducing substances, urobilinogen, bile, and microscopic examination.

Pathology: All animals were autopsied and the tissues were fixed in 10 per cent formalin or Bouin's solution. The tissues were stained with hematoxylin and eosin.

RESULTS

Acute Toxicity

Single oral doses of 0.5 to 1.5 gm. per kg. were tolerated by mice and rats without causing any outward signs of toxicity (table 1). However, within five to ten minutes after the administration of larger doses, signs of restlessness, running movements, and ataxia were noted. In most instances the stage of excitement was rapidly followed by depression. A dose of 3.0 gm. per kg. was lethal for 30 per cent of the mice and rats. Deaths appeared to result from respiratory failure and usually occurred within the first forty-eight hours following drug administration.

Chronic Toxicity

Rats: All rats which received daily doses ranging from 0.15 to 2.5 gm. per kg. survived the ninety-day period of drug administration (table 2). Doses of 0.31 to 0.62 gm. per kg. were well tolerated, and no outward signs of toxicity were noted during the ninety-day period. Above this level, there was a slight to moderate depression in the growth of the rats. On the other hand, rats which received the lowest level of 0.15 gm. per kg. gained more weight than the corresponding control animals. This appeared to be due to an increased consumption of food at this level. The hematologic and biochemical studies in these animals revealed no significant abnormalities. The pathologic findings are reported in a later section of this communication.

TABLE 2

CHRONIC TOXICITY OF PYRAZINAMIDE IN RATS

NUMBER OF RATS	DOSE	MORTALITY	AVERAGE WEIGHT OF GROUP*	WEIGHT INCREASE†
	gm. per kg.	*per cent*		*per cent*
10	0	0	198	100
10	0.15	0	242	122
10	0.31	0	223	113
10	0.62	0	188	95
10	1.25	0	179	90
10	2.5	0	151	76

* After ninety days of treatment.
† Controls set at 100.

Dogs: The animals which received doses of 0.25 to 0.5 gm. per kg. daily for the ninety-day period showed no outward signs of toxicity. The two dogs on the 1.0 gm. per kg. dose developed anorexia and vomited at irregular intervals shortly after treatment was initiated. One of the dogs on this level also had a transient erythematous rash on both ears and all four legs, which subsided approximately one month after onset despite continued therapy. However, the hair remained shabby and ruffled. In view of their moribund condition, the animals on the 1.0 gm. per kg. level were sacrificed after twenty-one and fifty-eight days of dosing, respectively. As will be described in detail below, the animal in which the values for hepatic function were altered also had pathologic changes in the liver. The hematologic and biochemical findings noted in all of the dogs were essentially normal except for the dog with the rash which received the 1.0 gm. per kg. level. This animal had a transient leukocytosis and an increased erythrocyte sedimentation rate during the time that the rash was present. In this same animal, the alkaline phosphatase began to rise after forty-two days of treatment and continued to increase until it reached a level of 14 to 16 Bodansky units shortly before death. At this time the icteric index was also elevated to 9.6, although there was no clinical evidence of jaundice. Other chemical tests of hepatic function showed no change. Shortly before the animal was sacrificed, the carbon-dioxide combining power of the plasma fell to 23 volume per 100 ml. without a significant change in the pH of the plasma.

HISTOPATHOLOGIC OBSERVATIONS

Tissue alterations were noted only in the liver and kidney.

Liver: The most severe changes occurred in the liver of one dog which received 1.0 gm. of pyrazinamide per kg. per day for a fifty-eight-day period. There was marked zonal necrosis of the liver cord cells involving the pericentral and periportal areas, principally the latter (figure 1). These necrotic areas formed interlacing bands of necrotic tissue which in places, principally beneath the capsule, conglomerated to present, grossly and microscopically, the picture of massive hepatic necrosis. The lymph channels in the portal areas were dilated and were readily seen on gross inspection of the surfaces of the liver and gallbladder. This

FIG. 1. Histologic section of subcapsular zone of liver, showing massive necrosis in right half of field and interlacing bands of necrosis, principally periportal, in left half of field. The dilated vessels are lymphatics. (Hematoxylin-eosin).

was the animal that revealed the elevated alkaline phosphatase and icteric index. The second dog on the 1.0 gm. per kg. per day level for only twenty-one days showed no abnormal changes in the liver. The livers of the two dogs which received 0.5 gm. per kg. per day for the eighty-day period were somewhat friable, and the cytoplasm of the cord cells was thinned. The lymphatics in the serosa of the gallbladder and in the portal areas were dilated in one of these 2 animals. The livers of the dogs on the 0.25 gm. per kg. level and the livers of all the rats were essentially normal.

Kidneys: The kidneys of one dog on the highest level for twenty-one days revealed a moderate number of gray-white streaks in the inner one-third of the medulla. Histologically, these streaks were irregular bluish-red, principally amorphous masses in endothelial-lined channels, probably lymphatics. A few of these masses were coarsely granular and others were suggestively lamellated. None of the other dogs revealed these streaks grossly, but, histologically, a few were present in the second dog on the 1.0 gm. level and in both dogs on the 0.5 gm. level. The kidneys of the dogs on the lowest level were normal in all respects. There were no changes in the kidneys of rats except in an occasional animal at the highest level of 2.5 gm. per kg. for ninety days. In these animals many fine vacuoles were observed in the epithelium of the proximal convoluted tubules. Moreover, the nuclei of the epithelial cells of the loop of Henle and of the distal convoluted tubules were small and compact, and the cytoplasm was vacuolated.

Other organs: There were no structural changes noted in the heart, lung, brain, testes, stomach, small and large intestine, pituitary, gallbladder, bone marrow, urinary bladder, lymph nodes, trachea, aorta, adrenals, pancreas, prostate, striated muscle, spleen, thyroid, parathyroid, skin, or diaphragm.

MISCELLANEOUS OBSERVATIONS

Findings on examination of the urine were normal throughout the study. There was no evidence of abnormal urobilinogen or bile excretion despite the advanced liver changes noted on autopsy.

Respiration and arterial pressure in cats: Pyrazinamide had no apparent effect on the blood pressure or respiration when given to cats in doses of 200 mg. per kg. intraperitoneally or 400 mg. per kg. orally. Observations were made during a five-hour period, thus allowing sufficient time for absorption of the material. When a fine suspension was injected slowly in the leg vein, doses of 30 or 60 mg. per kg. produced a rapid fall in blood pressure. The respiration increased in rate and depth as the blood pressure fell, but both returned to normal within sixty minutes following the injection.

DISCUSSION

According to Solotorovsky and associates (3), pyrazinamide shows antituberculous activity in mice at doses of approximately 0.25 gm. per kg. Campagna and co-workers (9) reported good results in human pulmonary tuberculosis following the administration of 3.0 gm. of pyrazinamide daily given concurrently with 4.0 mg. of isoniazid per kg. In the present study, 0.25 gm. of pyrazinamide per kg. daily for an eighty- to ninety-day period produced no evidence of toxicity in rats or dogs. Moreover, rats tolerated amounts as large as 1.25 gm. per kg. twice daily for a ninety-day period, and the only untoward effect observed was a slight suppression in the growth of the treated rats. The results show, however, that the drug is capable of causing severe toxic reactions in dogs when administered in doses of 1.0 gm. per kg. daily for a period of three to eight weeks. The earliest untoward effect in dogs was the occurrence of vomiting at irregular intervals during the early period of treatment. Except for one dog which developed a compensated acidosis shortly before death, the vomiting was never severe enough to cause alterations in the acid-base balance or the state of hydration. The most significant toxic effect was the occurrence of necrosis of the liver cord cells in the one dog which received 1.0 gm. per kg. for a fifty-eight-day period. Prior to death, there was evidence of liver dysfunction in this dog, as noted by the rise in the icteric index and alkaline phosphatase levels. The occurrence of liver damage in man during the administration of pyrazinamide-isoniazid or pyrazinamide alone has been reported by several groups of investigators (5, 8, 9, 12). The dog may offer an experimental approach for studies designed to learn more about the nature of the damage following pyrazinamide administration and to study methods for preventing this damage.

SUMMARY

Pyrazinamide was administered in single and repeated doses to mice, rats, and dogs. The chronic toxicity studies in rats and dogs extended over a ninety-

day period. In general, pyrazinamide was well tolerated by all species. Doses of 0.25 and 0.5 gm. per kg. were tolerated by dogs for a ninety-day period without evidence of toxicity. A level of 1.0 gm. per kg. produced intermittent vomiting in the 2 dogs fed this level and death after twenty-eight and fifty-eight daily doses, respectively. Prior to death, the alkaline phosphatase and icteric index of one dog treated for fifty-eight days were elevated and, at necropsy, gross and microscopic evidence of liver damage was present.

SUMARIO

La Toxicidad de la Piracinamida

La piracinamida fué administrada a dosis únicas y repetidas a ratones, ratas y perros. Los estudios de la toxicidad crónica en ratas y perros abarcaron un período de noventa días. En general, la piracinamida fué bien tolerada por todas las especies de animales. Dosis de 0.25 y 0.5 gm. por kg. de peso fueron toleradas por los perros durante un período de noventa días sin signos de intoxicación. Un régimen de 1.0 gm. por kg. produjo vómitos intermitentes en los 2 perros que recibieron esa concentración y la muerte al cabo de veintiocho y cincuenta y ocho dosis diarias, respectivamente. Antes de la muerte, la fosfatasa alcalina y el índice ictérico de un perro tratado durante cincuenta y ocho días estaban elevados y, en la autopsia, observáronse signos macro y microscópicos de lesión hepática.

RESUME

La toxicité de la Pyrazinamide

La pyrazinamide a été administrée à doses simples et à doses répétées à des souris, rats et chiens. Les études sur la toxicité du traitement continu chez le rat et le chien se sont poursuivies pendant une période de quatre-vingt-dix jours. D'une manière générale la Pyrazinamide a été bien tolérée par toutes les espèces. Des doses de 0.25 et 0. 5 g par kg ont été tolérées par le chien pendant une période de quatre-vingt-dix jours sans aucunes réactions de toxicité. Chez les 2 chiens recevant cette dose, une concentration de 1 g par kg a provoqué des vomissements intermittents et ensuite la mort au bout de vingt-huit et cinquante-huit doses quotidiennes, respectivement. Avant la mort, l'indice ictérique et la phosphatase alcaline du chien traité pendant cinquante-huit jours étaient élevés et, à l'autopsie, des signes macroscopiques et histologiques d'altération du foie étaient présents.

Acknowledgment

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STUDIES ON THE USE OF A HIGH DOSE OF ISONIAZID[1, 2]

I. Toxicity Studies

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INTRODUCTION

An outstanding property of isoniazid is its virtual lack of significant toxicity when used in conventional doses. Side effects, by their very inconspicuousness, have been a minor part of most clinical reports. Except for idiosyncrasies or allergies, reactions appear to be transient. Tabulations of drug toxicity have appeared (1, 2), but generally statistical analysis is difficult because of the nature of the complaints and the varied approaches of the observers. Those that have been recorded include restlessness, insomnia, muscle twitching, headache, vertigo, constipation, urinary retention, and subtle changes in behavior. Psychosis and convulsions are more significant phenomena. In connection with these, electroencephalographic abnormalities have been induced by isoniazid in higher doses (3), and fatal status epilepticus has occurred in one person who received 3 mg. of isoniazid per kg. daily and who had previously suffered from epilepsy (4).

The commonly used dose of isoniazid, 3 to 5 mg. per kg. per day, originally was chosen principally on the basis of experiments on dogs and mice. As a result of toxicity studies on these animals, higher doses have been considered inadvisable in humans. It has also been suggested that higher doses would exaggerate the otherwise minor side effects seen with the drug. Little is known, consequently, about the therapeutic effects of larger doses of isoniazid in human tuberculosis. The interest of the present writers in this question was sharpened by observations which suggested that high doses of isoniazid might be therapeutically superior to the doses ordinarily used. It was observed by Schmidt and Jolly (5) that a high incidence of cures could be obtained in monkeys with naturally acquired tuberculosis through the use of daily doses of 20 mg. of isoniazid per kg., given for a period of five months. In contrast, their control series, which received only 5 mg. per kg. per day, showed a therapeutic response roughly comparable to that of humans. They noted that isoniazid was well tolerated in daily doses up to 80 mg. per kg. and, thus, that it was considerably less toxic for the monkey than for the dog (7).

On the hypothesis that human tuberculosis might also show more rapid clearing with such high doses of isoniazid, and also that significant toxicity in hu-

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mans at the same time might not be increased in severity or frequency, a study of toxic and therapeutic effects of 20 mg. of isoniazid per kg. per day was planned. In this report, observations with respect to the toxic effects are described.

PLAN OF INVESTIGATION

Case Material

All cases studied were patients with active tuberculosis at Dunham Hospital or the Cincinnati General Hospital. Selection of patients for the study was arbitrary, but included the bulk of the previously treated patients admitted to either hospital during the period from February 10 to May 15, 1953. Also included was a group of patients who had been receiving standard doses of isoniazid for varying times preceding the study. Patients with epilepsy, or with peripheral neuritis from nutritional deficiency, alcoholism, or other causes, were usually excluded on admission because of possible predisposition to toxicity. A few patients, however, with neuritis of this sort were deliberately included for specific study of this possibility.

Methods

Toxic effects were first informally explored. Fifty patients who had never received antituberculous chemotherapy, hereafter referred to as *Group A*, and 39 other patients who had previously received such treatment (including isoniazid), referred to as *Group B*, were studied. The patients in *Group B* had been receiving isoniazid, 200 to 300 mg. (3 to 5 mg. per kg.) per day, plus streptomycin or dihydrostreptomycin, 1 gm. daily or twice weekly. The patients in both groups were given 6 to 24 mg. of isoniazid per kg. per day, which was continued for a period of at least four months unless toxicity supervened. Streptomycin was also started or continued in either of the doses mentioned above. The patients were observed closely for clinical toxicity, and random examinations of peripheral blood, urine, and liver function were performed. In none of these laboratory tests was significant abnormality demonstrated.

OBSERVATIONS

Of the patients in *Group A*, clinical toxicity was almost nil during the treatment period, except for anorexia and nausea in 4 patients, and for the appearance of peripheral neuritis in 9 during the fourth to eighth weeks. A more detailed discussion of these phenomena is given below. *Group B* patients fared similarly. Three exhibited gastric intolerance, and 23 of the 39 developed neuritis. Three patients of this group died. One man developed massive hemoptysis, not apparently related to therapy. The cases of the other 2 are more fully described.

Illustrative Case Reports

Case 1. A 55-year-old man with far advanced pulmonary tuberculosis was first given isoniazid, 250 mg. per day, on June 26, 1952. On February 12, 1953, this was raised to 500 mg. per day, and on February 20, 1953, to 800 mg. per day (20 mg. per kg.). The dose was increased because the patient had been getting alarmingly worse and in the hope that it might be helpful on an empiric basis. On February 22, 1953, he suddenly collapsed and told a friend, "This is the end." When seen by the physician he was markedly cyanotic, in shock, and

obviously dying. Autopsy was not obtained. It was believed that death was due to myocardial infarction.

Case 2. A 61-year-old man was admitted November 10, 1952, with a diagnosis of acute miliary tuberculosis. Lumbar puncture revealed a normal fluid, with no cells; protein, 22 mg. per 100 ml., sugar 48 mg. per 100 ml., and a culture negative for tubercle bacilli. The treatment started on the day of admission was streptomycin, 2 mg. daily, and isoniazid, 300 mg. daily. The clinical response was excellent.

On February 2, 1953, aching in the chest and left arm were noted. Examination and an electrocardiogram were normal. The symptoms were transient. On February 16, the dose of isoniazid was increased to 600 mg. daily (10 mg. per kg.), on the twenty-first to 900, and on the twenty-fourth to 1,200 mg. daily (20 mg. per kg.). This regimen was well tolerated except for eructations for a few days. Eosinophilia of 28 per cent appeared at this time; the total leukocyte count was 11,700. On March 13, 1953, epigastric distress became severe, with fullness and pain, and isoniazid was reduced to 600 mg. daily, with prompt relief of symptoms.

At approximately this time, numbness, tingling, and sense of swelling appeared in the fingers. Vibratory sense was absent in all extremities, and the tendon reflexes were not present. Specific hypesthesia could not be demonstrated. Nicotinamide, 300 mg. daily, was started on March 26, without relief. On March 30, isoniazid was reduced to 300 mg. daily. By this time the neuritis was severe and a source of great concern to the patient. On April 6, strength was greatly reduced, and all modalities of sensation were diminished peripherally. There appeared to be fixation of the joints of the hand by spasm or contracture of the forearm muscles. Tendon reflexes were slight, with positive snout and sucking reflexes. Plantar responses were flexor. Isoniazid was discontinued on April 13, streptomycin was reduced to 1 gm. daily, and PAS was added. The patient became disoriented on April 17, with incoherent mumbling. Catatonic flexibility was noted, and suddenly a generalized seizure occurred. Lumbar puncture was performed, with normal findings, including a culture negative for tubercle bacilli. Convulsions recurred, and he remained deeply comatose. The blood pressure was well maintained, and an electrocardiogram revealed abnormalities of hypokalemia. Cheyne-Stokes respiration appeared, with increasing periods of apnea, and he was placed in a respirator.

A few hours after the initial convulsive seizure, neurologic examination showed nystagmus to the left, with hyperactive deep reflexes on the left. Flaccidity was generalized. A serum potassium concentration, reported later, was 3.5 mEq. per liter. Eight grams of potassium chloride were given in the ensuing twelve hours, without appreciable clinical effect. The patient was removed from the respirator and remained comatose.

Consistent neurologic findings were not found. On April 23, pyridoxine was begun to a total of 1,000 mg. intramuscularly over the next four days. On April 21 he appeared to be awake and tried to speak, but was unable to express himself. Fever appeared, and he died on April 28. Autopsy was not obtained.

Comment: Generalized cerebral disease was present in this patient, the nature of which is obscure. The temporal relationship to drug therapy is striking, but similar phenomena did not occur in any other patients treated at this dosage level.

Chance Selection Study

After several weeks of preliminary observations, a formal study was put into effect in which patients who had received no previous chemotherapy were assigned by a chance selection method for treatment on one of several regimens. One regimen consisted of 20 mg. of isoniazid per kg. daily, with or without streptomycin. This regimen was used for the treatment of 27 patients designated

Group C. Therapy was continued for at least two months (60 to 360 days; average, 151 days) unless toxicity supervened. Ten of the 27 patients developed peripheral neuritis, and 4 developed gastric intolerance; there were no other significant symptoms of drug toxicity in the entire group.

Peripheral Neuritis

Clinical manifestations: The occurrence of peripheral neuritis was not recognized until a rather fully developed picture was present in a number of patients. It is interesting to note, however, that the first instance observed in this community occurred in a woman who was receiving only 300 mg. of isoniazid daily. The manifestations of isoniazid peripheral neuritis follow a pattern which is characteristic enough that early detection is now the rule. It must be realized that suggestibility is a prominent factor in provoking and sustaining some of the symptoms of peripheral neuritis and that this is of particular importance in patients at rest in a tuberculosis hospital. Moreover, it should be emphasized that truly objective evidence of nerve involvement is absent in the earliest stages. For these reasons, the present writers avoided direct questioning regarding symptoms of neuritis but waited for volunteered complaints by the patients. Significant leads were then followed with careful questioning.

Symptoms usually began with a feeling of tingling in the fingers or toes, likened by some to "pins and needles" or to the limb's "going to sleep." Numbness was occasionally a presenting complaint. Weakness and stiffness of the joints were mentioned by some. These were early manifestations and were usually not associated with definite physical findings. If isoniazid was stopped at this stage, reversal was usually seen within a few days or weeks.

Later symptoms, if the drug was continued at high doses, consisted of tenderness of the calves, a more burning type of sensation, and increasing weakness of the affected limbs. Abnormal findings at the time included exaggerated or decreased reflexes at the knee and ankle, hypalgesia and hypesthesia, periosteal and muscle tenderness, and decreased or absent perception of vibration in the distal parts. Symptoms persisted for months when they were allowed to progress to this stage, even if isoniazid was stopped altogether. In all but 2 of the patients, no residual disability was present at the end of twelve months following discontinuance of the drug. Late findings in these 2 patients include burning feet, atrophy, fasciculations, and continued weakness.

The incidence of neuritis in previously untreated patients who received doses of isoniazid ranging from 6 to 24 mg. per kg. per day is given in figure 1 (*Groups A* and *C*). There was a definite correlation between dose and the incidence of the reaction; 44 per cent of the patients who received the highest dosage (16 to 24 mg. per kg. per day) showed neuritis. Not included on this graph, nor in the data above, are 3 patients who received 3 to 5 mg. of isoniazid per kg. per day who developed neuritis in the twenty-sixth to twenty-eighth week of therapy. Comparison of these patients with those in *Groups A* and *C* reveals some tendency for neuritis to appear later with lower doses and earlier with higher doses (figure 2).

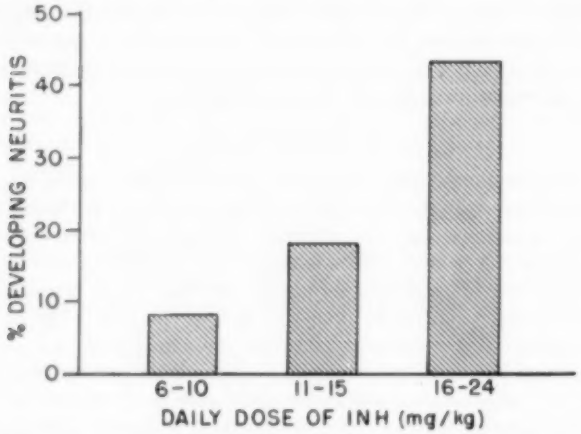

FIG. 1. Incidence of peripheral neuritis in patients who received no previous isoniazid according to dose of isoniazid given. The total number of patients with neuritis was nineteen.

Late development of muscle contracture: Of interest in those who developed neuritis is a late finding which was not associated with protracted residual neuritis or correlated with the original severity of the neuritis. Muscle contracture of the hands and feet with limited function was seen in 5 patients, including *Case 2* above. The contractures have been persistent and progressive, and marked deformity has resulted in 2 patients in whom standard doses of isoniazid had been resumed. The causal association of this phenomenon with isoniazid is not established, however, and requires further observation. Of interest is the fact that 2 of these patients never received more than 3 to 5 mg. of isoniazid per kg. per day.

Influence of previous low-dose isoniazid therapy: Previous isoniazid in standard dosage appears to condition the patient to an earlier onset of neuritis after the dose is raised, and possibly also causes a higher incidence of the phenomenon. Thus, 7 patients who had previously received 3 to 5 mg. per kg. daily developed neuritis within a week after increasing the daily dosage of isoniazid to higher levels. Of a total of 37 patients who previously received isoniazid, neuritis appeared in 23 (62 per cent) after the daily dose was increased.

There was no correlation with anemia or other associated condition. The presence of malnutrition and frank avitaminosis on admission to the hospital did not predispose to isoniazid neuritis, nor did pre-existing neuritis, for example, caused by alcoholism or diabetes, become worse with therapy.

Gastric Intolerance

Anorexia, at times with nausea and vomiting, appeared in 11 patients (10 per cent) of the total group of 116 patients and necessitated reduction in dosage in

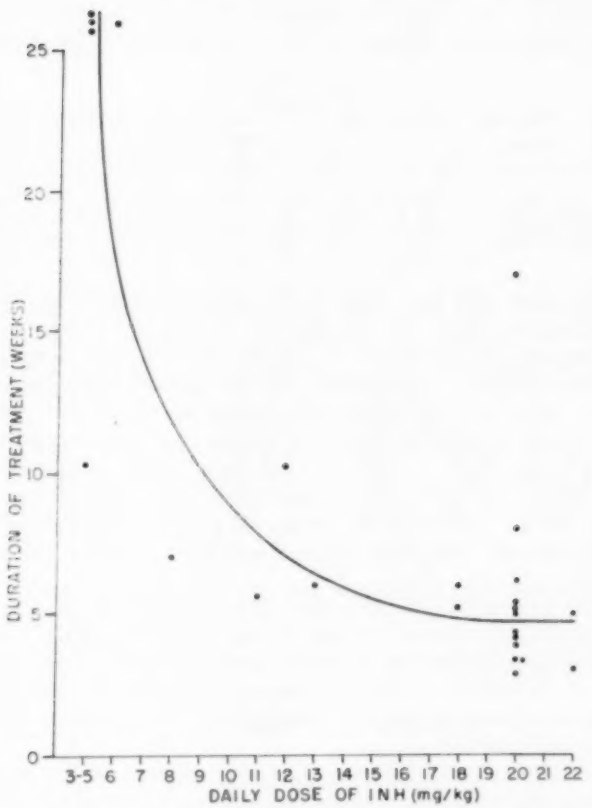

FIG. 2. Time of onset of isoniazid neuritis according to dose given

all. Ten of these patients were receiving 19 to 20 mg. per kg. per day; the other became nauseated on 10 mg. per kg. per day. The onset was usually within a few days after starting medication, but was delayed for three to six weeks in 2 cases. Reduction of the dose by one half uniformly provided relief. The administration of pyridoxine, 300 mg. daily, was effective in completely relieving nausea in one patient, and the high dose of isoniazid was maintained. Pyridoxine was ineffective in 2 other patients.

Clinical Laboratory Findings

Peripheral blood counts: Complete blood counts were performed every one or two weeks for the first six weeks, followed by another at three months. Changes in the hemoglobin level with therapy were largely upward, the only exceptions being explainable on the basis of hemoconcentration at the time of admission. The average level of hemoglobin on admission was 11.0 gm. and, at a point

TABLE 1

EFFECT OF ISONIAZID (20 MG. PER KG. PER DAY) ON HEMOGLOBIN AND TOTAL LEUKOCYTE COUNT

(25 patients)

	WEEK OF THERAPY							3 MONTHS
	0	1	2	3	4	5	6	
Mean hemoglobin (gm.)	11.0	10.5	10.9	11.4	10.7	10.8	11.3	13.0
Mean leukocyte count	8,800	9,100	9,800	9,400	9,000	9,500	8,100	9,200

three months later, 13.0 gm. (table 1). The transient fall during the first few weeks described by others was not observed. No correlation was observed between anemia and the appearance of neuritis. Total leukocyte counts showed no significant variations from normal. The average values for the group were perhaps somewhat elevated before and throughout treatment, including the count taken at three months. Occasional unexplained leukocytosis to the level of 15,000 cells was observed which was not accompanied by any unusual findings in the differential counts. These leukocyte elevations were sporadic, followed no pattern, and the average of the counts did not vary significantly (table 1). Eosinophilia was observed frequently and amounted to 28 per cent in one patient (*Case 2*).

Urinalyses: Examination of the urine, performed on the same schedule as the blood counts, showed no consistent or significant abnormality in any case. Isolated specimens occasionally showed a trace of albumin. Specifically, glycosuria was not induced in nondiabetic patients or altered in diabetics. Therapy with high doses of isoniazid did not complicate diabetic control, nor did it cause changes in insulin dosage.

Hepatic function: Tests of hepatic function consisted of thymol turbidity, thymol flocculation, cephalin flocculation, and zinc turbidity tests, total lipid, and serum bilirubin. Bromsulfalein retention was not measured unless a significant abnormality appeared in the rest of the tests. The liver function tests were obtained on the same schedule as the blood counts. Abnormal values for cephalin flocculation or zinc turbidity appeared in 9 of 17 patients in whom adequate studies were made. These changes were not persistent; they appeared mostly during the second and third weeks and then subsided. In only 4 patients were abnormal values found on more than one occasion.

Isoniazid concentrations: Fifty-four determinations of the concentration of isoniazid in the blood were made in the high-dose group (*Group C*), using the method of Kelly and Poet (7). Blood specimens were withdrawn two and one-half hours after a dose. Values of up to 14.0 γ per ml. were noted, the average of all being 6.7 γ. Fifty-four similarly obtained specimens from patients who received 300 mg. daily showed values ranging from 0.9 to 5.1 γ per ml., the average being 2.3 γ. In the high-dose patients there was no correlation between the concentrations of isoniazid present in the blood and the appearance of neuritis. Nevertheless, this does not exclude a possible correlation between circulating

uncombined isoniazid and neuritis, for it has been shown (8) that much of the drug measured by the procedure used is in the form of the acetyl derivative.

DISCUSSION

It has been anticipated by some investigators that high doses of isoniazid would prove to be excessively toxic. The less important symptoms which appear with standard doses would logically be expected to be exaggerated with increased dosage. The present study indicates that manifestations which can be loosely grouped under the heading of cerebral or autonomic stimulation are no more apparent with the high-dose regimen than when a schedule of 3 to 5 mg. per kg. per day is used. The present investigators have been impressed by the inconspicuousness of such complaints, regardless of dose. It is believed that avoidance of systematic direct question of the patients in connection with restlessness, insomnia, and similar symptoms accounts in part for this seeming discrepancy from previous experience. It is concluded that a high dose of isoniazid (20 mg. per kg. per day) is well tolerated by the tuberculous patient, except for occasional gastric intolerance and for the complication of peripheral neuritis. Whether the instance of possible toxic encephalopathy represented a consequence of the high level of the dosage or might have occurred from the use of isoniazid in customary dosage cannot be determined at present.

The 3 deaths which occurred are of concern, for they bore some relationship to the increase in dosage of isoniazid. One death, which followed a massive pulmonary hemorrhage, appeared to be clearly unrelated to drug toxicity. The circumstances of the other 2 deaths have been recited in detail. In the opinion of the present writers, only one of these 2 fatalities (*Case 2*) could reasonably be attributed to the isoniazid therapy.

These data are to be compared with the experience from the use of comparably high doses of isoniazid in dogs. Doses of 17.5 mg. or more per kg. per day were found to be toxic for the dog by Rubin, Hassert, Thomas, and Burke (8). Central nervous system dysfunction, including convulsions, and jaundice (with marked fatty degeneration of the liver) resulted when this dose was given to two animals, the symptoms appearing after two to four weeks. Benson, Stefko, and Roe (9) also reported "slight liver pathology" in dogs which received 5 and 20 mg. per kg. per day. In the 32 patients who received 16 to 24 mg. per kg. per day in the present study, one developed convulsive seizures with subsequent encephalopathy and 14 developed neuritis. The evidence of hepatic dysfunction was much less definite. Changes in liver function tests appeared in approximately one-half of the 17 patients studied, but were found on repeated testing in only 4 patients. While these changes were real, they were transient in every case. Thus, a daily dose of 17.5 mg. per kg. in the dog and 20 mg. per kg. in man apparently has a high degree of neural toxicity, but significant hepatic toxicity was not observed at this dosage in the patients even though it had been observed in dogs. The difficulty in applying animal toxicologic data to primates has been emphasized by Schmidt, Hughes, and Hoffman (10).

Peripheral neuritis as a side effect of isoniazid therapy has been repeatedly

encountered (11–16). Its relationship to the metabolism of pyridoxine has been reported from this laboratory (17). Pyridoxine deficiency may appear with isoniazid therapy, due to increased excretion of pyridoxal, and possibly also because of the inactivation of pyridoxal in the tissues by isoniazid. Neuritis may be explained on this basis, since neuritis identical to isoniazid neuritis may be seen in pyridoxine deficiency in humans induced by desoxypyridoxine, a metabolic antagonist of pyridoxine (18). It has been shown in a significant number of patients that pyridoxine will prevent isoniazid neuritis if given from the start of therapy (17).

Another metabolic mechanism correlating with the appearance of isoniazid neuritis has to do with the manner in which isoniazid is metabolized. Hughes, Biehl, Jones, and Schmidt (19) have shown that neuritis appeared in those patients excreting more free isoniazid, less acetylated isoniazid, and another breakdown product, as yet unidentified. The latter substance tended to appear only in those patients who developed peripheral neuritis. The relationship of pyridoxine to this mechanism has not been established.

Other isonicotinic acid hydrazide derivatives also produce neuritis. 1-Isonicotinyl, 2-isopropyl hydrazide (iproniazid) (1) and glucuronolactone isonicotinyl hydrazone (20) are specific examples. It is not clear, however, whether or not these compounds are stable *in vivo* or are broken down to isoniazid. Pyridoxal isonicotinyl hydrazone has not been studied for neurotoxic effects. It is quite insoluble, however, and is unstable at the low ranges of pH attained within the stomach. These characteristics discourage clinical trials. This compound is said, at the same time, to possess significant antituberculous qualities *in vivo* and *in vitro* (21).

Indirect evidence tends to confirm the adverse effects of high doses of isoniazid in epileptics. While only one case of clear-cut increased seizure frequency in an epileptic is on record, Reilly and associates have shown a lowered seizure threshold in nonepileptic schizophrenics who received more than 20 mg. per kg. as an isolated intramuscular dose. One of their patients convulsed after 35 mg. per kg. (3). It appears at present that the hazard of seizure exacerbations is significant in epileptics while receiving isoniazid. Pyridoxine will prevent seizures due to thiosemicarbazide (22), and it has been suggested but not confirmed that pyridoxine similarly would protect against isoniazid-induced seizures (3). Pyridoxine deficiency, either dietary (23) or resulting from desoxypyridoxine (24), may induce seizures.

The administration of pyridoxine with high doses of isoniazid does protect against neuritis and, thereby, furnishes a technique whereby one may use such doses over long periods of time without the hazard of neuritis. At the same time, pyridoxine does not appear to interfere with the chemotherapeutic response to isoniazid in so far as comparisons are possible among treated patients. The patients in the series described elsewhere (17) included many who were also receiving pyridoxine, 50 to 450 mg. per day, with 20 mg. of isoniazid per kg. per day and streptomycin. During a period of six months, tubercle bacilli disappeared from the sputum of these patients at least as quickly as of those in a compara-

ble control group. Pyridoxine, therefore, does not grossly interfere with demonstrable therapeutic effectiveness of isoniazid.

Whether or not prolonged high-dose isoniazid therapy will prove superior to conventional doses is uncertain at present and will be the subject for further study. It is not to be construed on the basis of this paper that the addition of pyridoxine to other regimens involving isoniazid would be harmless. While no adverse clinical effects have been noted from its use in conjunction with high doses of isoniazid, it is not to be assumed that, if used with the conventional dose of isoniazid, for example, pyridoxine would not in some way detract from the antibacterial effects of isoniazid.

SUMMARY

Toxic effects of isoniazid, using doses in excess of 5 mg. per kg. per day, are described in a group of 116 patients with pulmonary tuberculosis. Consistent toxicity was not found; side effects encountered were peripheral neuritis and gastric intolerance. Peripheral neuritis was increasingly common with increasing dosage up to 24 mg. per kg. per day; 44 per cent of those who received 16 to 24 mg. per kg. per day developed neuritis. Three patients died during the course of the study, one possibly from toxic encephalopathy due to isoniazid. Another death occurred suddenly from an unknown cause but was not believed to represent a consequence of high-dose isoniazid therapy. The third death was clearly unrelated to the administration of the isoniazid. Gastric intolerance was seen in 10 per cent of the group; most cases occurred in those who received 20 mg. per day. No significant toxic effects on the peripheral blood, kidneys, or liver were noted.

SUMARIO

Estudios de los Efectos de Una Dosis Alta de Isoniacida

Descríbense los efectos tóxicos de la isoniacida, a dosis superiores a 5 mg. por kg. al día, en un grupo de 116 tuberculosos pulmonares. No se observó toxicidad constante, efectos subsidiarios notados fueron neuritis periférica e intolerancia gástrica. La neuritis periférica aumentó cada vez más al elevarse la dosis hasta 24 mg. por kg. al día; 44 por ciento de los que recibieron de 16 a 24 mg. por kg. al día manifestaron neuritis. Tres enfermos fallecieron durante el estudio, uno de ellos posiblemente de encefalopatía tóxica debida a la isoniacida. Otra muerte ocurrió repentinamente de causa desconocida, pero al parecer no fué consecuencia de la isoniacidoterapia a dosis altas. La tercera merte no guardaba a las claras relación con la administración de isoniacida. Observóse intolerancia gástrica en 10 por ciento del grupo; la mayor parte de los casos correspondió a los que recibieron 20 mg. por kg. al día. No se notaron efectos tóxicos de importancia sobre la sangre periférica, los riñones o el hígado.

RESUME

Etudes sur l'emploi d'une dose élevée d'Isoniazide

Description des effets toxiques de l'isoniazide à doses dépassant 5 mg/kg par jour dans 116 cas de tuberculose pulmonaire. La toxicité n'a pas été observée de façon constante; les réactions secondaires constatées consistaient en névrite périphérique et intolérance gastrique. La fréquence de la névrite périphérique augmentait en même temps que la dose

augmentait jusqu'a atteindre 24 mg/kg par jour; 44 pour cent des sujets qui recevaient de 16 à 24 mg/kg par jour ont présenté de la névrite. Trois malades sont morts au cours de l'étude, le décès de l'un d'entre eux paraît être dû à une encéphalopathie toxique imputable à l'Isoniazide. Un autre décès subit de cause inconnue a eu lieu, mais il n'est pas considéré comme une conséquence de l'isoniazide-thérapie. Le troisième décès était nettement sans relation avec l'administration d'isoniazide. L'intolérance gastrique a été observée dans 10% des cas étudiés; la plupart se produisant chez les sujets recevant 20 mg/kg par jour. Il n'a été constaté aucun effet toxique important sur le sang périphérique, le rein ou le foie.

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THE SIGNIFICANCE OF RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS TO ISONIAZID[1]

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INTRODUCTION

Bacterial susceptibility tests are usually performed at infrequent intervals on cultures of *Mycobacterium tuberculosis* obtained from sputum and other infected materials. In order to ascertain whether such infrequency gives an adequate picture of the incidence of bacterial susceptibility to isoniazid, tests were carried out on cultures obtained twice weekly from patients who received isoniazid alone or in combination with other drugs. Between March, 1952, and July, 1953, 31 patients with pulmonary tuberculosis were so studied at the Brompton Hospital.

Although it is now generally recommended that, like streptomycin, isoniazid be administered with a second chemotherapeutic agent in the treatment of pulmonary tuberculosis, recent work indicates that this analogy may not be as close as it appeared initially (1). The observation that some patients continue to improve in spite of the isolation of isoniazid-resistant bacilli from their sputum presented another reason for carrying out this study. It should be stressed, however, that until this problem is clarified, isoniazid should not be given alone in the routine treatment of tuberculosis.

MATERIALS AND METHODS

Of the 31 patients with pulmonary tuberculosis studied, 25 were suffering from a recent exacerbation of their chronic disease, and 24 had cavities. There were only 6 patients with recent acute disease, so that a comparison of clinical and roentgenographic progress with drug-susceptibility studies usually was unrewarding. All were inpatients receiving isoniazid for the first time; only 13 had previously received streptomycin or para-aminosalicylic acid (PAS). One patient was given isoniazid for only two and one-half months, but all of the others received the drug continuously for at least three months. The standard dose of isoniazid was 200 mg. daily, but 4 patients were given 300 mg. daily. Eighteen patients received isoniazid alone for the first three months and, in 7 cases, this was continued for varying periods, in one instance for eleven months. The drug combinations used during and after the first three months of treatment are shown in table 1.

At the commencement of the study, all of the patients had tubercle bacilli in the sputum; twenty-four-hour samples of sputum were thereafter examined twice weekly by microscopy and culture. Laryngeal swabs were taken twice weekly from 7 patients in the later stages of their treatment when they produced no sputum; every fourth week, two

[1] From the Brompton Hospital for Diseases of the Chest, London, England.

TABLE 1

THE DRUG COMBINATIONS USED DURING AND AFTER THE FIRST THREE MONTHS OF TREATMENT

DRUGS EMPLOYED	FIRST 3 MONTHS OF TREATMENT	SECOND 3 MONTHS OF TREATMENT AND SUBSEQUENTLY
Isoniazid alone	18	8
Isoniazid plus streptomycin	8	10
Isoniazid plus PAS	1	1
Isoniazid plus streptomycin plus PAS	4	3
Streptomycin plus PAS	—	6
No chemotherapy	—	3
Total patients under treatment	31	31

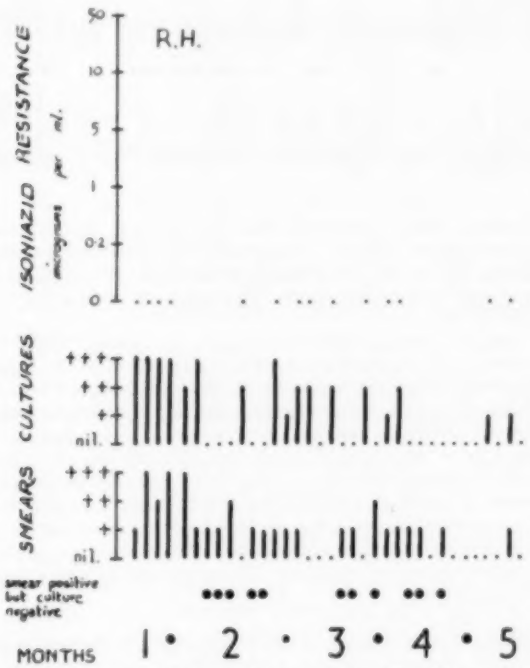

FIG. 1. Twice-weekly bacteriologic results in a patient (R. H.) persistently producing isoniazid-susceptible cultures.

gastric lavage samples were obtained in place of the laryngeal swabs. All specimens were "concentrated," using 10 per cent trisodium phosphate solution, and then inoculated onto two Löwenstein-Jensen slopes. Susceptibility to isoniazid was determined for each strain of *M. tuberculosis* isolated, and susceptibility to streptomycin and PAS was measured if these drugs were being administered. The methods used were those employed in the Medi-

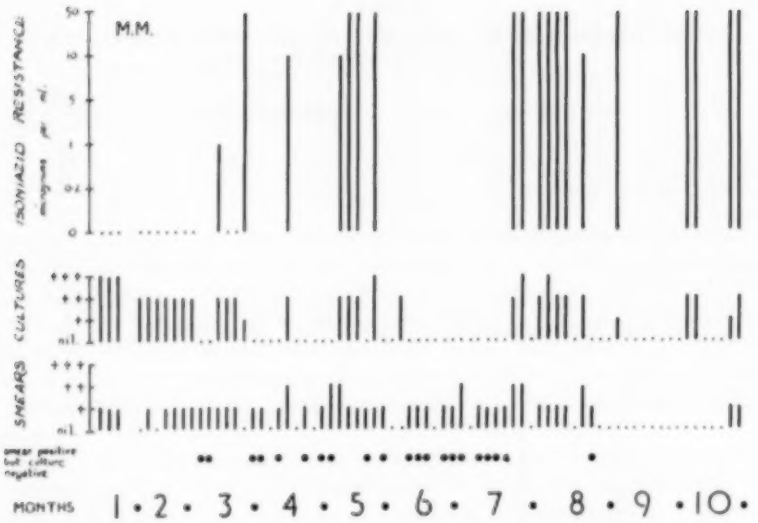

FIG. 2. Twice-weekly bacteriologic results in a patient (M. M.) persistently producing highly resistant cultures.

cal Research Council (MRC) isoniazid trial (2). A total of 1,653 twenty-four-hour sputum samples were examined in this way, in addition to 92 laryngeal swabs and 31 gastric lavage samples. The period of continuous observation of individual patients varied between three and eight months; the number of twice-weekly specimens from each patient varied between 24 and 84.

The clinical progress, roentgenographic changes, microscopy, culture, and isoniazid-susceptibility results were recorded graphically for each patient. In figures 1, 2, and 3, acid-fast bacilli present in smears from concentrated specimens are recorded as nil, plus (less than 10 bacilli per one-sixth-inch objective field), 2-plus (10 to 20 bacilli per field), and 3-plus (more than 20 bacilli per field). Culture readings are plotted as nil (less than 20 colonies), plus (20 to 100 colonies), 2-plus (innumerable discrete colonies), and 3-plus (confluent growth). Isoniazid resistance is recorded in terms of the maximum concentration of drug present in the medium permitting growth (more than 20 colonies) of the culture isolated; a susceptible culture (inhibited by 0.2 γ of isoniazid per ml.) is plotted with a small dot. The occurrence of a smear-positive, culture-negative specimen is recorded with a bold dot at the bottom of the figure. Time intervals are given in months from the start of isoniazid treatment.

When discussing results, bacterial susceptibility will be expressed in terms of the third report of the MRC isoniazid trials (2).

> This defines "sensitive" strains of *M. tuberculosis* as those which show no growth (less than 20 colonies) on the 0.2 γ per ml. or higher concentrations of isoniazid after four weeks' incubation; also those which grow after four weeks' incubation on 0.2 γ per ml. but not on 1 γ per ml., provided that the H37Rv strain of *M. tuberculosis* shows the same or a greater degree of growth on 0.2 γ per ml. "Doubtfully resistant" strains

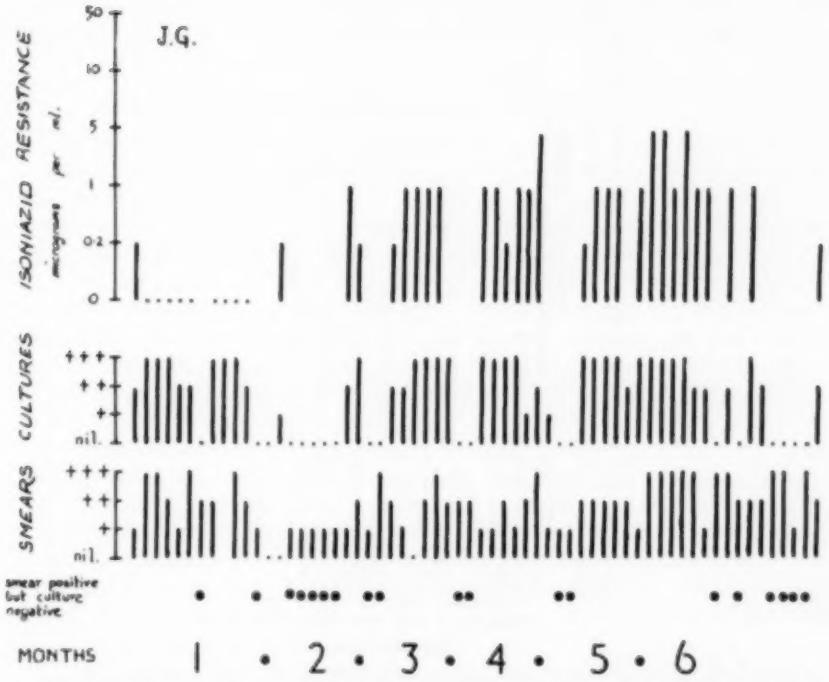

FIG. 3. Twice-weekly bacteriologic results in a patient (J. G.) yielding cultures of varying degrees of resistance.

are those which grow (20 or more colonies) on Löwenstein-Jensen medium containing 0.2 γ of isoniazid per ml., but not on a medium containing 1 γ per ml., provided that the H37Rv strain shows a lesser degree of growth on the 0.2 γ per ml. slope. "Resistant" strains are defined as those which grow (20 or more colonies) on the 1 γ per ml. slope.

RESULTS

A comparison of monthly, fortnightly, weekly, and twice-weekly drug-susceptibility test data revealed certain limitations of infrequent examinations (figure 4). Patients with resistant strains of *M. tuberculosis* were identified six times by monthly examinations and seventeen times by twice-weekly tests, although in 5 of these a resistant culture was isolated only once.

Times of Appearance of Resistant Strains

The time of emergence of resistant organisms in the 18 patients on isoniazid alone has varied between 43 and 198 days from the start of treatment when the first susceptible culture was isolated (average, 86 days). There is some evidence

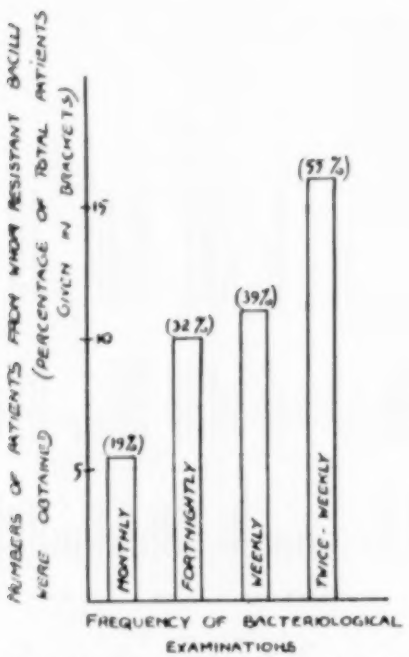

FIG. 4. Detection of isoniazid-resistant strains of *M. tuberculosis* in 31 patients receiving isoniazid alone or in combination with other drugs. The numbers of patients yielding resistant strains at some time during the investigation are recorded from the twice-weekly bacteriologic examination results. From these data also, the numbers which would have been recorded had the examinations been at weekly, fortnightly, and monthly intervals after the start of treatment have been recorded. The criterion of bacterial resistance for this comparison is the isolation of a culture which grew (at least 20 colonies) in the presence of 1 γ or more of isoniazid per ml.

to support the observation that resistant strains were isolated earlier from patients who were acutely ill (Medical Research Council, 1952); in 4 patients whose pretreatment erythrocyte sedimentation rate exceeded 50 mm. in one hour (Westergren), resistant strains appeared after 60, 43, 53, and 72 days, respectively (average, 57 days). In 7 patients in whom the one-hour erythrocyte sedimentation rate was always less than 50 mm., resistant strains appeared after an average of 106 days from the start of treatment and the first drug-susceptible culture. There is also some evidence that those patients who produced early resistant cultures were more likely to continue to do so than those who produced them late in treatment. Of 3 patients showing earliest resistant cultures (after 43-, 53-, and 53-day intervals, respectively), 2 developed organisms with sustained resistance to 50 γ of isoniazid per ml., while cultures from the third patient were first resistant to only 1 γ of isoniazid per ml. and later were

drug susceptible. In the 3 patients whose resistant strains were most delayed (after 118, 160, and 198 days) and were found only once, all later cultures reverted to complete susceptibility.

Pattern of Bacterial Resistance to Isoniazid

The 18 patients who received isoniazid alone may be grouped into three categories according to the way in which their cultures behaved when grown in the presence of isoniazid.

The drug-susceptible group: The drug-susceptible group comprised 4 patients with disease of moderate extent and one with only a minimal lesion. Three of them did not yield a positive culture after the first month of treatment, so that the later susceptibility results are unknown. The other 2 patients, who received isoniazid for five months, produced organisms which remained completely susceptible throughout, except for one isolated finding of resistance to 0.2 γ of isoniazid per ml. The behavior of a representative member of this group is illustrated in figure 1.

The drug-resistant group: The drug-resistant group consisted of 3 patients with extensive bilateral disease. Strains of *M. tuberculosis* grown from each patient on at least ten different occasions were resistant to an isoniazid concentration of 50 γ per ml. The duration of isoniazid therapy was three and one-half, seven, and eight months, respectively. In one patient, the emergence of resistant organisms preceded clinical and roentgenographic deterioration; the second patient showed slight improvement; and the third appeared unchanged. The behavior of this group is exemplified in figure 2.

Group showing fluctuating resistance: In the group with cultures of fluctuating resistance, there were 10 patients, 9 of whom had disease of moderate extent while the tenth one had advanced disease. Isoniazid was given for two and one-half, three, three, three and one-half, four, four and one-half, five, eight and one-half, eight and one-half, and eleven months, respectively. The resistance to isoniazid of organisms isolated from this group was, for the most part, intermediate in degree. Three of the patients yielded only one resistant culture, while the other 7 each produced between two and forty resistant cultures. Highly resistant cultures, i. e., to 50 γ of isoniazid per ml., were encountered only four times, and these were from 3 patients. Figure 3 is a representative graph from one of the group showing fluctuating resistance.

Isolation of Susceptible Cultures After Resistant Ones

Drug-susceptible cultures recurred during the study of 7 patients from whom resistant cultures had previously been obtained (figure 5); monthly or fortnightly tests might well have failed to demonstrate these. Two reverted from resistance to 1 γ or more of isoniazid to 0.2 γ of isoniazid per ml., and 5 to complete susceptibility. In 3 of the 7 patients, this "reversion" was based on a single culture of diminished resistance; in the other 4 patients, several such cultures were isolated. The "reversion" was temporary in 2 patients but, in the other 5, the last known positive cultures were still susceptible. In 6 of the 7, the "reversion" was

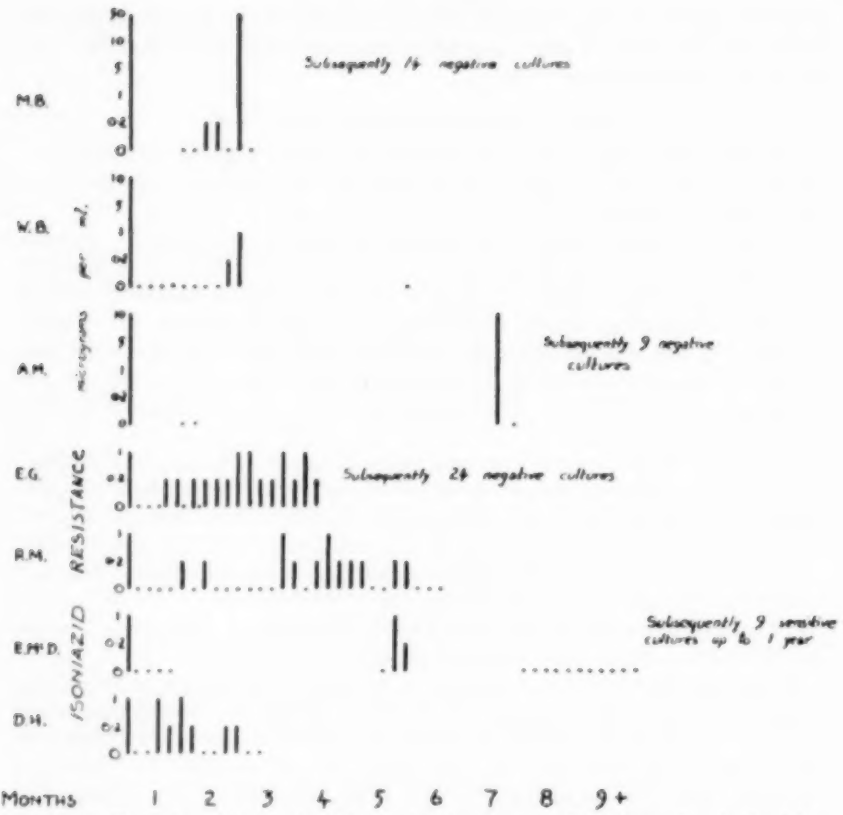

FIG. 5. Examples of "reversion" in isoniazid-resistance. Culture results obtained by twice-weekly examination of 6 patients on isoniazid alone are recorded; results from the seventh patient are given in figure 4. Details of a patient (D. H.) on isoniazid, streptomycin, and PAS are included.

noted while the patient was still receiving isoniazid. "Reversion" of isoniazid resistance was also noted in patients receiving all three drugs.

Failure of Bacterial Growth on Culture Medium

Specimens from 29 patients at some time showed organisms on microscopy which failed to grow on culture. In 13 patients, the failure rate was 25 per cent, and one patient showed this phenomenon in twenty-six of forty-four sputum samples (59 per cent).

DISCUSSION

In this study, frequent drug-susceptibility tests provided additional information about the emergence of resistant bacilli in those patients with limited dis-

ease and in those from whom cultures of tubercle bacilli were difficult to obtain. In 9 of 10 patients with extensive disease, however, monthly examinations of sputum yielded as much information as twice-weekly tests. Since this investigation takes note of only two-sevenths of all the twenty-four-hour sputum samples from each patient, it is quite possible that the incidence of resistant strains isolated would be still higher than that estimated if all available sputum samples were examined. The current opinion that bacterial resistance is more common in patients with cavities may merely reflect the readiness with which positive cultures are obtained.

The incidence of isoniazid-resistant strains of *M. tuberculosis* in "closed" lesions receives little mention in the literature. Of the 31 patients examined twice weekly, 19 yielded strains at some time which grew on the 0.2 γ slope; 10 of these patients subsequently produced strains which were completely susceptible, while the remaining 9 produced organisms of greater resistance. Of 12 patients whose organisms grew in the presence of 1 γ of isoniazid per ml., 6 later produced only susceptible strains, while the other 6 yielded strains more resistant than before. Bacilli from only 3 of the 6 patients whose organisms became increasingly resistant grew in the presence of 5 γ per ml. of isoniazid, and in all 3 this tolerance later increased with ultimate growth on the 50 γ per ml. slope. In all, 7 patients eventually produced organisms which grew on the 50 γ slope, and 5 of these maintained their high level of resistance. From only one of the other 2 patients was a fully susceptible strain subsequently isolated, and from the other an organism was isolated which grew in the presence of 1 γ (but not more) of isoniazid per ml.; this, however, occurred only in isolated cultures. Strains resistant to 1 γ of isoniazid per ml. were often replaced by completely susceptible organisms, but, in this series, once a strain resistant to 5 γ or more of isoniazid per ml. was isolated, it was more likely to recur than those of lower degrees of resistance.

It has recently been stated that "the clinically significant level of bacillary resistance to isoniazid is not yet determined" (3). The present studies lead to the belief that "resistance" might better be defined for clinical purposes as failure of 5 γ of isoniazid per ml. to inhibit growth of the culture of *M. tuberculosis* under examination; the present accepted level is probably too low. Since many clinicians tend to stop isoniazid as soon as bacterial resistance is reported, it seems wise to report resistance only when this is likely to be a constant feature, namely, when a level of 5 γ is attained. The acceptance of 5 γ rather than of 1 γ of isoniazid per ml. as a critical level in the development of bacterial isoniazid tolerance might encourage the development of strains resistant to streptomycin or PAS. There is no published work to suggest that this is a serious hazard; the present series is too limited to give decisive information on this point. Of 9 patients treated with isoniazid only, and who produced bacilli growing on the 1 γ slope, only one showed definite clinical and roentgenographic evidence of deterioration, which became manifest two months after the isolation of resistant cultures. A chemotherapeutic response has been demonstrated in guinea pigs infected with strains of *M. tuberculosis* which will grow in the presence of 1 γ of isoniazid per kg. of body weight (4). On the other hand, some maintain that resis-

tance to 0.2 γ is often significant; from a group of 27 patients who received isoniazid, 8 were found who developed such resistance associated with a rise in bacillary sputum counts and often with unfavorable roentgenographic changes (5). However, as Coates and associates point out, their patients represent a selected group of far advanced chronic cavitary disease. It is doubtful whether their findings are generally applicable.

Fluctuating levels of isoniazid resistance in serial cultures of *M. tuberculosis* indicate the isolation, from time to time, of strains of varying degrees of tolerance to the drug from the total population of tubercle bacilli present in sputum. These fluctuating levels of resistance may not reflect changes in the organisms isolated from one lesion, but the isolation of strains from different foci of infection. There was no means of establishing which factor was the more important in this group, since no data from resection or autopsy material were available. In this series, the possibility of one or more active lesions being closed off by lung collapse therapy could be excluded. From 2 patients, each suffering from what appeared to be a single lesion, susceptible strains followed the isolation of resistant ones; it is possible that this was due, in these 2 cases, to changes in the bacterial populations of these lesions. "Reversion" of previously resistant organisms has been noted on a number of occasions (6–9); it is also known to occur *in vitro* (4, 10).

During this investigation the high incidence of "failured" cultures which occurred in certain patients was substantiated by a carefully controlled comparison with patients giving a high positive culture rate and by using both 10 per cent trisodium phosphate and 4 per cent sodium hydroxide for "concentrating" the specimens. The findings made it clear that the cause of "failed culture" was inherent in the specimens and not in any particular concentration method. It has been suggested that growth of tubercle bacilli may be prevented by the presence of antibacterial drugs in sputum samples sent for culture (11). Failure of growth may, however, occur independently of antimicrobial therapy (12). In 5 of the patients in this study, it was not possible to culture tubercle bacilli from pretreatment specimens of sputum in which acid-fast bacilli could be demonstrated on microscopy. While the significance of the "failed culture" remains uncertain, its recognition is of some importance, and it behooves the clinician still to consider both the microscopic and cultural results of sputum examination.

SUMMARY

The frequency of sputum examinations influences the probability of finding isoniazid-resistant strains in patients under treatment with this drug.

Examinations made at frequent intervals show that bacterial resistance of strains isolated from serial cultures is not necessarily a permanent phenomenon once it appears. After isolation of a strain resistant to isoniazid, later strains are often found to be susceptible.

The isolation of a single culture resistant to 1 γ of isoniazid per ml. should not be the sole reason for discontinuing isoniazid.

Arguments are put forward in favor of revising the definition of a clinically significant level of bacterial resistance to isoniazid. The isolation of a culture of *M. tuberculosis* growing in the presence of 5 γ of isoniazid per ml. indicates a degree of bacterial resistance which is likely to recur in later isolates.

SUMARIO

La Importancia de la Resistencia del Mycobacterium Tuberculosis a la Isoniacida

La frecuencia con que se hacen los exámenes del esputo afecta la probabilidad de descubrir cepas isoniacido-resistentes en los enfermos en vías de tratamiento con dicha droga.

Exámenes verificados a plazos frecuentes demuestran que la resistencia bacteriana, una vez aparecida, de las cepas aisladas de cultivos en serie no constituye forzosamente un fenómeno permanente. Después de aislar una cepa resistente a la isoniacida, las cepas subsiguientes resultan a menudo susceptibles.

El aislamiento de un solo cultivo resistente a 1 γ de isoniacida por ml. no debe representar la única causa para discontinuar la isoniacida.

Ofrécense argumentos en pro de una revisión de la definición de un índice clínicamente importante de la resistencia bacteriana a la isoniacida. El aislamiento de un cultivo de *M. tuberculosis* que forma colonias en presencia de 5 γ de isoniacida indica un índice de resistencia bacteriana que probablemente recurrirá en las cepas aisladas después.

RESUME

La signification de la résistance du Mycobacterium tuberculosis à l'Isoniazide

La frequence de l'examen des crachats augmente les chances de découvrir les souches isoniazide-résistantes chez les malades en cours de traitement par ce composé.

Les examens répétés fréquemment montrent que la résistance bactérienne des souches isolées de cultures en série n'est pas nécessairement un phénomène constant lorsqu'il a fait son apparition. Après isolement d'une souche résistante, les souches ultérieures peuvent souvent se montrer sensibles à l'isoniazide.

L'isolement d'une seule culture résistante à 1 γ d'isoniazide par ml. ne doit pas constituer la raison unique de la suppression de l'isoniazide.

Présentation d'arguments en faveur d'une révision de la définition du degré cliniquement significatif de la résistance bactérienne à l'isoniazide. L'isolement d'une colonie de *M. tuberculosis* se développant en présence de 5 γ d'isoniazide par ml. indique un degré de résistance bactérienne susceptible de se reproduire dans les isolats ultérieurs.

Acknowledgments

The writers wish to express gratitude to the medical and nursing staff of the Brompton Hospital for their cooperation during these studies. Acknowledgment is also made to the technical staff of the Bacteriology Department and to Mr. D. F. Kemp (Photographic Department, Institute of Diseases of the Chest) for their help. The work was made possible by a grant from the Research Fund of the Hospitals for Diseases of the Chest, London.

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THE AMOUNT OF PYRIDINE NUCLEOTIDES (COENZYMES I AND II) IN BLOOD IN EXPERIMENTAL TUBERCULOSIS BEFORE AND DURING ISONIAZID TREATMENT[1]

JORMA PÄTIÄLÄ

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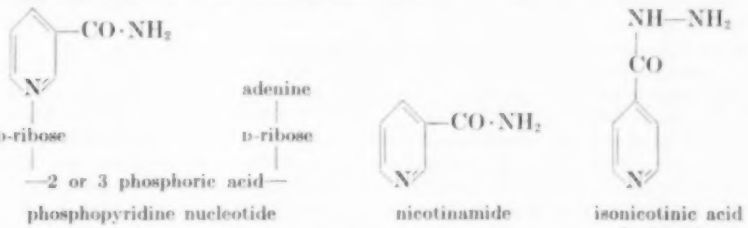
INTRODUCTION

Pyridine nucleotides (PN) are coenzymes of certain dehydrogenases, i. e., they are prosthetic groups consisting of either two or three phosphoric acid molecules. With two phosphoric acid molecules, the coenzyme is known as diphosphopyridine nucleotide (coenzyme I or codehydrogenase I); with three phosphoric acid molecules, it is called triphosphopyridine nucleotide (coenzyme II or codehydrogenase II). Further components of pyridine nucleotides are ribose, adenine, and nicotinamide (the last-named being a very essential one). These pyridine nucleotides, of which the greater part is diphosphopyridine nucleotide, play an important role in the metabolism of cells and are, accordingly, almost universally to be found in the tissues of animals. Most cells, particularly in the liver, are able to synthesize these coenzymes. In the blood they are present entirely in the erythrocytes. According to recent investigations, reduced values of pyridine nucleotides are quite commonly obtained from blood in tuberculosis by a biologic method, as has been shown by Rossini (1), and by Kerppola and Pätiälä, who employed a chemical method on a more comprehensive material (2).

Isoniazid, which has a considerable antituberculous effect, is closely related to nicotinamide, the basic component of pyridine nucleotides. The difference is that the side chain in the pyridine ring has the para-position instead of the meta-position of the nicotinamide.

Accordingly, it seemed of interest to study the influence of isoniazid on the amount of pyridine nucleotides in laboratory animals. The purpose of the present investigation was to study the concentrations of pyridine nucleotides in the blood and, in a few cases, in the livers of animals.

The composition of pyridine nucleotides, nicotinamide, and isoniazid may be described by means of the following formulas:



[1] From the First Medical Department, University of Helsinki, Finland.

MATERIALS AND METHOD

Thirty guinea pigs, each weighing approximately 600 gm., were subcutaneously inoculated in the inguinal region with 0.1 mg. of virulent tubercle bacilli, strain H37Rv. The animals were divided into three groups of 10, two of which were administered 20 mg. (40 mg.) of isoniazid (Rimifon®)[2] per kg. daily by mouth. The drug was given by means of a gastric tube in 1 ml. of aqueous solution. The amount of blood that was needed for the determination of pyridine nucleotides, approximately 2.5 ml., was withdrawn by means of heart puncture under light ether narcosis. Only eight heart punctures of a total of ninety caused the death of the animal.

The method used for the determination of the total pyridine nucleotides in blood is a fluorometric method by Nora Levitas and associates (3). The alkali acetone condensation reaction for N_1-methylnicotinamide yields with both pyridine nucleotides a highly fluorescent product whose fluorescence intensity is about twice as high, per equivalent, as that of the *N*-methylnicotinamide derivate. It can be used for the quantitative determination of the total coenzymes in amounts as small as 1 γ, corresponding to 0.05 ml. of whole blood or 0.02 ml. of erythrocytes. The oxalated blood is deproteinized with trichloracetic acid, the condensation reaction is carried out on the filtrate, and the fluorescence is read in the fluorometer within ten minutes after filtration.

An internal standard of N_1-methylnicotinamide is employed and the results are calculated in terms of either diphosphopyridine nucleotide or nicotinic acid per ml. of erythrocytes, on the basis of the recovery value and of the simultaneously determined hematocrit. It is emphatically suggested that determinations of nicotinic acid or of the pyridine nucleotide content of whole blood are both useless and misleading unless the corresponding hematocrit readings are given.

Reagents and Apparatus

25 per cent trichloracetic acid
6N NaOH, prepared daily by dilution of the carbonate-free saturated solution (about 19 N)
6N HCl, any chemically pure grade of concentrated hydrochloric acid diluted 1:1 with water
Redistilled acetone, rendered free of fluorescent substances by distillation over $KMnO_4$
20 per cent KH_2PO_4 solution
The standard solution of N_1-methylnicotinamide containing 1 γ per ml.
The standard quinine sulfate solution containing 0.3 γ per ml. in 0.1 N H_2SO_4
Fluorometer
Cuvette tubes graduated at 10 ml.

Procedure

As it has been amply demonstrated that practically all of the nicotinic acid derivates are confined to the erythrocytes, the determination may be carried out on the whole blood without any loss of accuracy if hematocrit determinations are made.

By means of heart puncture, 2.5 ml. of blood are drawn and placed into a bottle containing a previously dried mixture of 3 mg. of ammonium oxalate and 2 mg. of potassium oxalate. This amount is sufficient for 2.5 ml. of blood without affecting the cell volume. The blood should be laked and precipitated simultaneously and as soon as possible because the coenzymes are rapidly destroyed upon hemolysis. If hemolysis has not occurred, the blood can stand for as long as twenty-four hours without loss of diphosphopyridine nucleotide.

The determination of hematocrit is carried out with 0.1 ml. of the oxalated blood.

Two milliliters of whole blood are added dropwise and with constant shaking into a

[2] Kindly supplied by Hoffmann-LaRoche, Inc., Nutley, New Jersey.

large test tube (20 by 180 mm.) or a 50 ml. round-bottomed centrifuge cup containing 2 ml. of 25 per cent trichloracetic acid and 6 ml. of water. The contents are well mixed by rapid inversion for one minute. The solution is allowed to stand for several minutes and is then centrifuged five to ten minutes at 3,000 r.p.m. and filtered through a 7 cm. No. 30 Whatman paper. The clear filtrate is stable and will not deteriorate for several days if kept in the refrigerator. This filtrate provides more than enough material for duplicate analyses.

Into the bottom of each of three graduated test tubes is measured 0.5 ml. of blood filtrate. To tube 1 is added 0.5 ml. of water; to tube 2 (the blank), 1 ml. of water; to tube 3, the recovery 0.5 of N_1-methylnicotinamide contained in 0.5 ml. of the working standard solution.

To tubes 1 and 3, 0.5 ml. of acetone is added and the contents are mixed. To each of the tubes is added 0.20 ml. of 6N sodium hydroxide directly into the solution, touching off the last drop near the surface of the solution and mixing the contents of each tube immediately after the addition of the alkali. The tubes are allowed to stand at room temperature for five minutes. To each tube, 0.3 ml. of 6N hydrochloric acid is added, mixing each tube at once after the addition. The tubes are immersed into a boiling water bath for two minutes, 1 ml. of 20 per cent potassium dihydrogen phosphate solution is added, and the contents are diluted with water to the 10 ml. mark and mixed. The fluorescence reaches a maximum immediately and remains stable in the daylight or in the dark for at least three days; therefore, it may be measured at any convenient time.

The intensity of the fluorescence of the compound obtained from blood filtrates by this method follows the same curves with respect to temperature and pH as those which are given by Huff and Perlzweig for *N*-methylnicotinamide.

Diphosphopyridine nucleotide of per cent purity gives a recovery value with blood filtrate of 15 divisions per microgram, while *N*-methylnicotinamide added to an aliquot of the same filtrate yields a value of 38 divisions per microgram in the Coleman model 12 fluorometer. This proportionality factor, 15/38 = 0.4, remains constant, so that, if recovery values other than 38 divisions per microgram of *N*-methylnicotinamide are encountered, a correct diphosphopyridine nucleotide value may be calculated from the above factor. However, it must be realized that this is a recovery value and is not the same as that obtained with the standard solution alone.

Example of calculation:—Hematocrit 42 per cent; dilution, 2 ml. of whole blood to 10 ml. of 0.5 ml. filtrate (= 0.1 ml. blood) taken for analysis; also 0.5 ml. of filtrate plus 0.5 γ of N_1-methylnicotinamide for recovery value.

Readings:

Blood filtrate	58 divisions
Blank	12 divisions
Recovery	78 divisions

58 − 12 = 46 divisions for 0.1 ml. of whole blood

78 − 58 = 20 divisions for 0.5 γ of N_1-methylnicotinamide

= 40 divisions for 1 γ N_1-methylnicotinamide

40 × 0.4 = 16 divisions for 1 γ of diphosphopyridine nucleotide

$\frac{46}{16} \times \frac{1}{0.1}$ = 29 γ of diphosphopyridine nucleotide per 1 ml. of whole blood

$\frac{29 \times 100}{42}$ = 68 γ of diphosphopyridine nucleotide per 1 ml. of erythrocytes

All fluorescent determinations are made by using the Beckman model "DU" Quartz-spectrophotometer.

Experiment I

In the first experiment, the pyridine nucleotide determinations were made in a control series of 10 animals which received no isoniazid treatment. Inoculation with tubercle bacilli was performed at the beginning of the experiment. Deter-

TABLE 1

PYRIDINE NUCLEOTIDE VALUES IN THE ERYTHROCYTES, THE WHOLE BLOOD, AND THE LIVER, AND THE HEMATOCRIT VALUES (*Experiment I*)

NUMBER	IN THE BEGINNING			AFTER 4 WEEKS			AFTER 6 WEEKS			
	Pyridine Nucleotide		Hematocrit	Pyridine Nucleotide		Hematocrit	Pyridine Nucleotide			Hematocrit
	Erythrocytes	Whole Blood		Erythrocytes	Whole Blood		Erythrocytes	Whole Blood	Liver	
	γ/ml.	γ/ml.		γ/ml.	γ/ml.		γ/ml.	γ/ml.	γ/gm.	
1	63.3	26.6	42.0	58.5	19.3	33.0	54.4	22.3		41.0
2	70.5	27.4	39.0	78.5	26.7	34.0	65.2	24.5		37.0
3	72.3	26.0	36.0	71.2	24.2	35.0	66.7	22.7		34.0
4	77.8	33.1	42.0	81.4	29.3	36.0				
5	77.9	32.7	42.0	76.3	26.7	35.0				
6	78.3	22.7	29.0	74.1	26.7	36.0	78.8	27.5	59.5	35.0
7	79.5	34.2	43.0	80.4	28.1	35.0	70.6	24.0		34.0
8	87.4	40.2	46.0	92.8	39.0	42.0	96.2	39.4	82.6	41.0
9	88.4	35.3	40.0	67.5	26.3	39.0	65.5	25.5		39.0
10	94.0	35.7	38.0	83.5	29.2	35.0	83.4	26.7	57.9	32.0
Average	78.9	31.3	39.7	76.4	27.5	36.0	72.5	26.5	66.6	36.6

minations of pyridine nucleotides were made at the same time and were repeated after four and six weeks, respectively. Two of the animals (Nos. 4 and 5) were killed during heart puncture and, hence, are represented by only two values. The results of the experiment may be seen in table 1; they are arranged according to the initial amount of pyridine nucleotide in erythrocytes. In addition, the table gives the pyridine nucleotide values for the whole blood, the hematocrit, and the values for the liver in the 3 animals which were examined. By way of further illustration, the pyridine nucleotide values for the erythrocytes are represented in figure 1.

It may be seen from the data in table 1 and figure 1 that, in the majority of the cases, the pyridine nucleotide values in erythrocytes show a falling tendency four and, especially, six weeks after the inoculation with tubercle bacilli. Six animals show values which are lower than the initial ones, and it is only in one case (No. 8) that a definite rise is seen. The average of pyridine nucleotide presents a corresponding fall: 78.9 γ per ml. to 76.4 γ per ml. to 72.5 γ per ml.

The fall of pyridine nucleotide in the whole blood follows the same pattern, and in these determinations also there was only one case of clear rise, while the average fell even more definitely: 31.3 γ per ml. to 27.5 γ per ml. to 26.5 γ per ml.

As it is important to know whether the pyridine nucleotide values, and especially the fall shown by them, have any definite relation to the extension of the tuberculous changes in the test animals, these were subjected to necroscopy. The spleen, liver, and lungs were examined histologically. The findings obtained

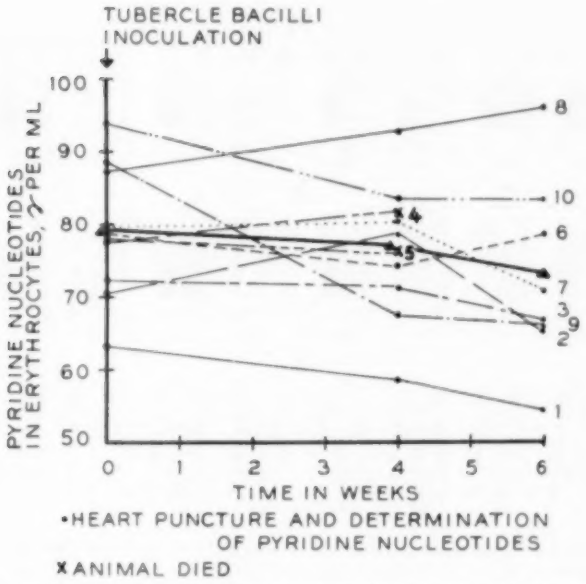


FIG. 1. Pyridine nucleotide values in erythrocytes according to *Experiment I*

were recorded by means of the Feldman-Hinshaw (4) method of representing tuberculous changes, with the one difference that, in the present investigation, only the three organs mentioned were included. The findings are to be seen in figure 2, in which the uppermost row of animals represents the control series (Group 1).

As may be seen from the diagram, the untreated control group shows tuberculous changes in all organs which were examined. A comparison of these changes with the corresponding pyridine nucleotide values obtained by the experiment reveals a clear conformity between them.

As a rule, the pyridine nucleotide values seemed to be in inverse proportion to the extension of the tuberculous changes, i. e., the greater the changes, the lower the values. For instance, in animal No. 9, whose pyridine nucleotide values had shown the relatively greatest fall, extensive tuberculous changes were present; whereas the tuberculous changes in animal No. 8, whose pyridine nucleotide value, unlike the rest, displayed a rise, are insignificant. In 3 animals (Nos. 6, 8, 10), the pyridine nucleotide value was also determined for the *liver* at the end of the experiment, whereby the following values were obtained: 59.5 γ per gm., 82.6 γ per gm., 57.9 γ per gm. By way of comparison, the pyridine nucleotide values were determined in the livers of 4 healthy animals which had lived under identical circumstances, the result being as follows: 134 γ per gm., 96.6 γ per gm., 104 γ per gm., and 107 γ per gm. Thus, it seems that the liver values of the test animals are considerably lower than those of the normal ones.

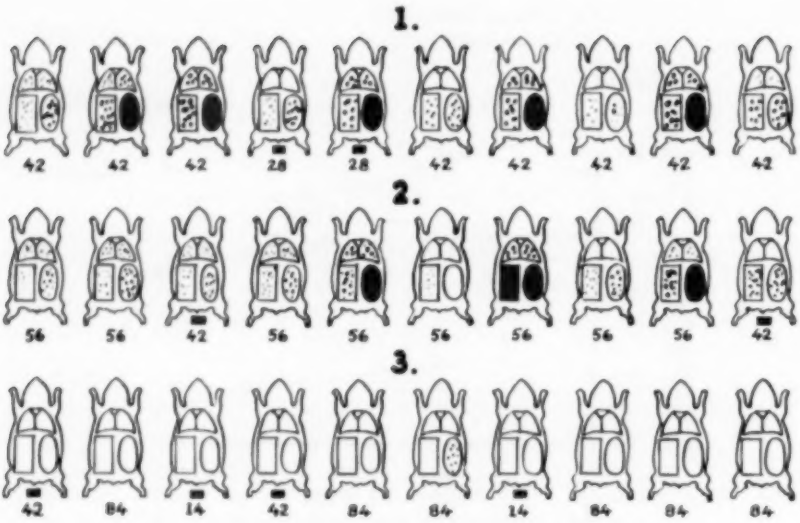


FIG. 2. Extent of tuberculosis in the liver, spleen, and lungs of the guinea pigs at necropsy in the three groups. Miliary and nodular lesions are represented by dots. Diffuse tuberculous involvement is indicated by complete blackening. The black rectangle below a figure indicates death of the animal during the experiment. The small numerals indicate the time in days from the beginning of the experiment to necropsy.

Statistical analysis revealed that the fall of pyridine nucleotide values after inoculation with tubercle bacilli was nearly significant in the erythrocytes and was significant in the whole blood (the change of a quantity, e. g., the amount of pyridine nucleotide in erythrocytes and in whole blood, is equal to the ratio of the difference between its final and initial values to its initial value). The difference between the hematocrit values was also almost of significance on statistical analysis, but in erythroctes the fall of pyridine nucleotide seemed to be more pronounced than one would expect in view of the decrease of hematocrit.

Experiment II

In the second experiment, as in the first, inoculation of the guinea pigs with tubercle bacilli was performed right in the beginning, together with a determination of pyridine nucleotide. Another determination of pyridine nucleotide was made after six weeks. Two animals were killed by heart puncture. The remaining 8 animals were given 20 mg. of isoniazid per kg. daily during a fortnight. After that, pyridine nucleotide was again determined. The animals were put to death and subjected to necroscopy as above. The results are to be seen in table 2 and figure 3.

In so far as the first six weeks are concerned, this experiment was identical with the previous one. The results also display a clearer conformity, for, even

TABLE 2

PYRIDINE NUCLEOTIDE VALUES IN THE ERYTHROCYTES AND THE WHOLE BLOOD, AND THE HEMATOCRIT VALUES (*Experiment II*)

NUMBER	IN THE BEGINNING			AFTER 6 WEEKS			AFTER 8 WEEKS		
	Pyridine Nucleotide		Hematocrit	Pyridine Nucleotide		Hematocrit	Pyridine Nucleotide		Hematocrit
	Erythrocytes	Whole Blood		Erythrocytes	Whole Blood		Erythrocytes	Whole Blood	
	γ/ml.	γ/ml.		γ/ml.	γ/ml.		γ/ml.	γ/ml.	
1	61.4	26.4	43.0	65.8	27.0	41.0	66.2	24.5	37.0
2	63.0	27.7	44.0	65.8	26.3	40.0	69.3	27.7	40.0
3	67.9	29.2	43.0	54.0	22.7	42.0			
4	72.0	31.6	44.0	69.5	27.8	40.0	82.9	38.1	46.0
5	73.0	31.4	43.0	79.5	28.6	36.0	82.0	32.0	39.0
6	82.5	27.2	33.0	91.7	36.7	40.0	89.0	36.5	41.0
7	83.0	36.5	44.0	62.2	19.9	32.0	78.0	27.3	35.0
8	83.0	33.2	40.0	69.1	28.3	41.0	91.3	38.3	42.0
9	86.2	36.2	42.0	85.4	26.5	31.0	92.5	33.3	36.0
10	95.8	35.4	37.0	90.4	32.5	36.0			
Average	76.7	31.4	41.3	73.3	30.6	37.9	81.4	32.2	39.5

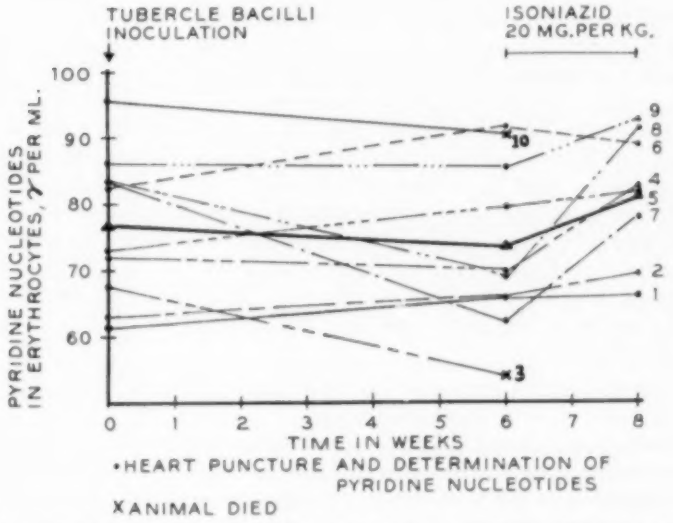


FIG. 3. Pyridine nucleotide values in erythrocytes according to *Experiment II*

TABLE 3

PYRIDINE NUCLEOTIDE VALUES IN THE ERYTHROCYTES, THE WHOLE BLOOD, AND THE LIVER, AND THE HEMATOCRIT VALUES (*Experiment III*)

NUMBER	IN THE BEGINNING			AFTER 2 WEEKS			AFTER 6 WEEKS			AFTER 10 WEEKS			AFTER 12 WEEKS			
	Pyridine Nucleotide		Hematocrit	Pyridine Nucleotide		Hematocrit	Pyridine Nucleotide		Hematocrit	Pyridine Nucleotide		Hematocrit	Pyridine Nucleotide			Hematocrit
	Erythrocytes	Whole Blood		Erythrocytes	Whole Blood		Erythrocytes	Whole Blood		Erythrocytes	Whole Blood		Erythrocytes	Whole Blood	Liver	
	γ/ml.	γ/ml.		γ/ml.	γ/ml.		γ/ml	γ/ml.		γ/ml.	γ/ml.		γ/ml.	γ/ml.	γ/gm.	
1	61.6	23.4	38.0	84.8	31.4	37	82.3	32.9	40.0							
2	64.4	29.6	46.0	67.5	29.3	44				53.1	22.3	42.0	77.9	31.9	122	41.0
3	80.3	32.0	41.0	107.9	47.3	42										
4	84.4	36.3	43.0	91.8	39.5	43										
5	88.5	39.8	45.0	107.8	45.2	42	104.2	42.7	41.0	96.8	39.8	41.0	99.5	45.7	207	46.0
6	92.5	29.6	32.0	142.3	55.5	39	126.5	51.6	40.0	87.0	37.4	41.0	110.3	49.6	257	44.0
7	96.2	39.4	41.0	97.9	33.3	34										
8	99.5	41.7	42.0	93.2	34.5	37				70.8	29.8	42.0	105.0	45.2	257	43.0
9	103.3	49.6	48.0	105.2	44.2	42				87.4	36.7	42.0	97.4	41.8	112	43.0
10	104.0	42.6	41.0	98.5	40.4	41	89.8	39.5	44.0	101.1	41.3	41.0	102.8	44.2	140	43.0
Average	87.4	36.4	41.7	99.6	40.1	40	100.5	41.7	41.2	82.5	34.5	41.5	98.6	43.0	182	43.3

in these circumstances, in most cases the values of pyridine nucleotide fell after inoculation of the animals with tubercle bacilli.

After the animals had been administered isoniazid, the pyridine nucleotide rose again in all animals except one. As a rule, the rise was very clear, especially in such animals as showed a greater than normal fall of pyridine nucleotide after inoculation with tubercle bacilli (Nos. 7 and 8). Animal No. 6 was different from the rest in so far as its pyridine nucleotide value still showed a slight fall after two weeks. After inoculation with tubercle bacilli, its pyridine nucleotide began to rise in contrast to the usual course of events. Necroscopy revealed that the tuberculous changes in this particular animal were relatively insignificant and limited to the spleen and the liver alone. After treatment with isoniazid, the average of pyridine nucleotide in the whole group rose from 73.3 γ per ml. to 81.4 γ per ml. in the erythocytes and from 30.6 γ per ml. to 32.2 γ per ml. in the whole blood.

Experiment III

In the third experiment, 10 animals were given isoniazid for a two-week period before they were inoculated with tubercle bacilli. The isoniazid was continued thereafter in the same dosage (20 mg. per kg. daily) for an additional six weeks and then the amount was doubled for a final two weeks, making ten weeks' chemotherapy in all. In the course of the experiment, heart puncture was performed five times: at the beginning and then after two, six, ten, and twelve weeks. Two animals died after the first puncture and another 2 after the

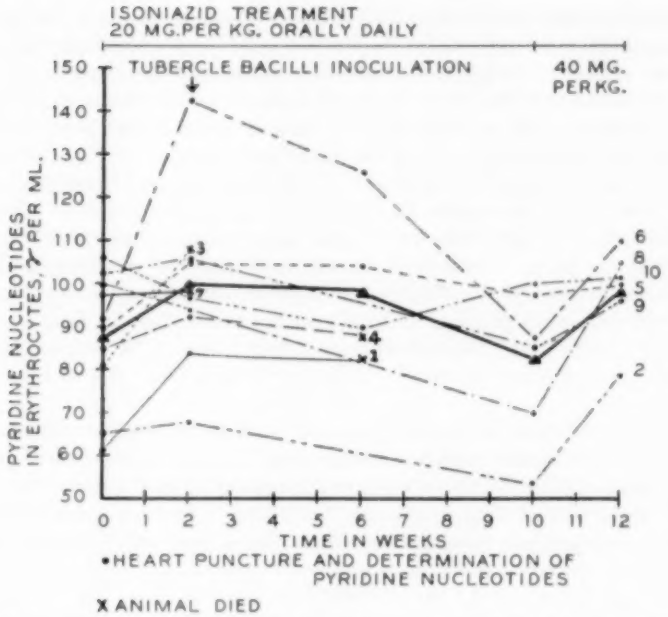


FIG. 4. Pyridine nucleotide values in erythrocytes according to *Experiment III*

second. The results of the experiment may be seen in table 3 and figure 4, as well as in the lowest line in figure 3.

A survey of the results shows that, for a period of two weeks, isoniazid caused an increase of pyridine nucleotides in the erythrocytes. The rise was seen in 8 animals; the average of the values was clearly higher than in the pretreatment period (87.4 γ per ml. against 99.6 γ per ml.), the values for whole blood being 36.4 γ per ml. and 40.1 γ per ml. After inoculation with tubercle bacilli, the pyridine nucleotide values invariably fell in the same way as in the previous experiment. When the values obtained eight weeks after inoculation, i. e., ten weeks after the beginning of the experiment, are compared with the initial values, it may be seen that both sets of figures are relatively close to one another.

In other words, a rise of pyridine nucleotides was brought about by isoniazid in the beginning of the experiment but, after inoculation with tubercle bacilli, the pyridine nucleotide concentrations fell to the level of the initial values and even below them. When the dose of isoniazid was doubled, the pyridine nucleotides invariably rose again in all test animals. Moreover, the average values showed a considerable rise: from 82.5 γ per ml. to 98.6 γ per ml. in the erythrocytes, and from 34.5 γ per ml. to 43.0 γ per ml. in the whole blood.

Necroscopy revealed tuberculous changes in only one animal in the whole series and, even in this case, they were quite insignificant. It is not clear from the results whether this was a consequence of the isoniazid administered during

the fortnight before inoculation with tubercle bacilli or of the administration of isoniazid after the inoculation. In the one animal (No. 6) which showed tuberculous changes, the pyridine nucleotide values rose to exceptionally high levels during the preinfection period of isoniazid administration at the beginning of the experiment but, eight weeks after inoculation with tubercle bacilli, the pyridine nucleotide values were at the initial level.

Determination of pyridine nucleotides in the liver was performed in all 6 animals at the end of the experiment. When the values obtained were compared with those obtained from the livers of normal animals (*see Experiment I*), it was found that the pyridine nucleotide values in the livers of the drug-treated infected animals were considerably higher, their average being 182 γ per gm. Although the values for pyridine nucleotide in the whole blood in *Experiment III* were also clearly higher than the corresponding values in the normal animals, the rise of the pyridine nucleotide values in the liver was comparatively more pronounced.

When the influence of isoniazid on the pyridine nucleotide values of the erythrocytes and whole blood was analyzed statistically, the increase of pyridine nucleotides in the erythrocytes, both in *Experiment II* and at the beginning as well as at the end of *Experiment III*, was found to be highly significant; the differences in the whole blood values were at the "almost significant" level.

DISCUSSION

In an earlier investigation carried out jointly by Kerppola and the present writer (2), the pyridine nucleotide values of erythrocytes and whole blood of 92 patients with tuberculosis were determined. The average value found in the erythrocytes was 54.0 γ per ml., and the average value in whole blood was 24.0 γ per ml. The corresponding average values in 42 normal persons was 67.7 γ per ml. and 28.7 γ per ml. Thus, the values for the pyridine nucleotides of erythrocytes and whole blood were clearly lower in tuberculous patients than in normal persons. In the present experiments, a corresponding fall of these values was likewise found in experimentally infected tuberculous guinea pigs and, moreover, the fall was the more pronounced, the more extensive the tuberculous changes. The values for pyridine nucleotides were also decreased in the livers of the tuberculous animals. Indeed, the latter phenomenon occurred in a higher proportion than in the blood.

Pyridine nucleotide values which had been caused to fall by tuberculosis rose, as a rule, under the influence of isoniazid and, indeed, within a relatively short period. Moreover, isoniazid effected a rise in the pyridine nucleotide values of normal guinea pigs.

When looking for an explanation of the facts, it should be borne in mind that nicotinamide, the central component of pyridine nucleotide, is structurally close to isoniazid, the difference occurring in the side chain of the pyridine ring. This would probably render it possible to replace nicotinamide by isoniazid in pyridine nucleotides. As isoniazid lacks the vitamin effect of nicotinamide, the result of the substitution of nicotinamide by isoniazid would seem to be a biolog-

ically inactive compound. The increased pyridine nucleotide values in blood and the remarkably high values found in the liver, as obtained after isoniazid treatment, seem to indicate that isoniazid is perhaps actually able to go into pyridine nucleotide molecules; in which situation, according to the method of determination employed above, it would behave like pyridine nucleotide. This hypothesis is corroborated by certain clinical observations. For example, pellagra, which is generally considered to be due to a deficiency of nicotinamide, has been manifested during isoniazid treatment (5, 6, 7). Moreover, some investigations into the metabolism of the tubercle bacilli have shown that the nicotinic concentration is very high within the bacterial cell (8, 9).

If the above hypothesis is correct, the results obtained with isoniazid in tuberculosis could be explained as the consequence of an inhibition of a large number of enzymatic reactions in which pyridine nucleotides are involved.

SUMMARY

Pyridine nucleotides (coenzymes I and II) were determined in the blood of experimentally infected tuberculous guinea pigs before and during isoniazid treatment. After the inoculation with tubercle bacilli, the values for pyridine nucleotides showed a tendency to fall. These reduced values rose again as a result of isoniazid treatment. Isoniazid effected a rise in the pyridine nucleotide values of normal animals as well. The increase in values for pyridine nucleotides seems to indicate that isoniazid is perhaps actually able to enter into the pyridine nucleotide structure. The resulting compound would obviously be biologically inactive in terms of pyridine nucleotide activity. If this hypothesis is correct, the results obtained with isoniazid in tuberculosis could be explained as a consequence of an inhibition of enzymatic reactions in which pyridine nucleotides are involved.

SUMARIO

La Cantidad de Nucleótidos de Piridina (Coenzimas I y II) en la Sangre en la Tuberculosis Experimental Antes de la Isoniacidoterapia y Durante la Misma

Se determinó la cantidad de nucleótidos de piridina (coenzimas I y II) en la sangre en la tuberculosis experimental de los cobayos antes de la isoniacidoterapia y durante ésta. Después de la inoculación de bacilos tuberculosos, los valores de dichos nucleótidos revelan tendencia descendente. Esos valores reducidos comenzaron invariablemente a elevarse de nuevo a consecuencia del tratamiento con isoniacida. La isoniacida logró igualmente un aumento en las cifras de nucleótido de piridina en animales de ensayo sanos. Esas cifras mayores parecen indicar que la isoniacida quizás sea en realidad capaz de convertirse en nucleótidos de piridina. El compuesto resultante sería a las claras inactivo biológicamente. Los resultados obtenidos con la isoniacida podrían, pues, explicarse como debidos a la inhibición de reacciones enzimáticas en que intervienen nucleótidos de piridina.

RESUME

La quantité de nucléotides pyridiniques (coenzymes I et II) dans le sang, dans la tuberculose expérimentale avant et pendant le traitement par l'isoniazide

Les nucléotides pyridiniques (coenzymes I et II) ont été déterminés dans le sang dans la tuberculose expérimentale du cobaye, avant et pendant le traitement par l'isoniazide.

Après l'inoculation par le bacille tuberculeux, les valeurs des nucléotides montrent une tendance à décroître. Dans tous les cas ces valeurs réduites ont recommencé à s' élever la suite du traitement par l'isoniazide. L'isoniazide a également provoqué une élévation des valeurs des nucléotides pyridiniques chez des animaux témoins bien portants. Ces valeurs accrues semblent indiquer que l'isoniazide est peut-être capable de pénétrer effectivement dans les nucléotides pyridiniques. Le composé résultant serait évidemment inactif du point de vue biologique. En conséquence, les résultats obtenus avec l'isoniazide dans la tuberculose pourraient donc être expliqués comme le résultat de l'inhibition des réactions enzymatiques dans lesquelles les nucléotides pyridiniques sont intéressés.

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STUDIES ON ISONIAZID AND TUBERCLE BACILLI[1,2]

I. The Isolation of Isoniazid-Resistant Mutants *in Vitro*

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INTRODUCTION

It has been observed (1) in isoniazid-treated patients that specimens of sputum which contained acid-fast rods on microscopy were often negative for *M. tuberculosis* on culture on oleic-acid–albumin medium (2), and less frequently negative on the American Trudeau Society egg-yolk-glycerin-potato medium (3). Similar observations have been reported by Knox and his associates (4) and by Mitchison and his associates (5). Therefore, an extensive investigation has been made in an effort to define the shortcomings of the semisynthetic type of medium containing serum albumin for the primary isolation of tubercle bacilli from pathologic materials. It was necessary, moreover, to integrate the studies at two distinctly different levels: growth requirements of tubercle bacilli, in general (6); and growth requirements specifically related to the emergence of isoniazid-resistant organisms.

The object of this paper is to present data concerning the incidence of isoniazid-resistant mutants of tubercle bacilli in predominately isoniazid-susceptible, parent populations by quantitative analytic techniques *in vitro*. Early during the course of these studies, it became evident that variations of certain components of the growth medium, not only influenced the apparent incidence of resistant mutants, but also permitted the identification of at least three different types of isoniazid-resistant mutants from virulent, isoniazid-susceptible strains of tubercle bacilli.

MATERIALS AND METHODS

Culture media: The composition of the basic type of medium used in these investigations was derived from a simultaneous study of the growth requirements of large numbers of strains of tubercle bacilli on primary isolation from human pathologic material, as described in a separate communication (6). The basic medium had the following composition:

KH_2PO_4	1.0 gm.
$Na_2HPO_4 \cdot 12H_2O$	6.3 gm.
L-glutamate (sodium salt)	0.5 gm.
Ammonium sulfate	0.5 gm.
Na_3 citrate $\cdot 2H_2O$	0.1 gm.
$MgSo_4 \cdot 7H_2O$	0.05 gm.
$CaCl_2 \cdot 2H_2O$	0.0005 gm.
$ZnSo_4 \cdot 7H_2O$	0.001 gm.

[1] From the National Jewish Hospital at Denver, Colorado, and the University of Colorado School of Medicine.

[2] This study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

$CuSo_4 \cdot 5H_2O$	0.001 gm.
Pyridoxine hydrochloric acid	1.0 mg.
Biotin	0.5 mg.

The above were dissolved, in the order given, in 1,000 ml. of distilled water and the pH was adjusted to 6.6.

Before autoclaving, Tween® 80 was added in final concentration of 0.05 per cent, and, after autoclaving, albumin (fraction V) was added in final concentration of 0.5 per cent, for dispersed cultures in liquid medium (Tween®-albumin medium).

Before autoclaving, agar was added in final concentration of 1.5 per cent, and, after autoclaving, bovine serum albumin or oleic-acid–albumin complex was added in final concentration of 0.1 per cent to 0.5 per cent for growth on solid agar medium.

To all media, after autoclaving, glucose was added (0.2 per cent to 0.5 per cent final concentration) from a concentrated solution previously autoclaved with 0.005M citric acid.

Various additions were made to this basal medium: inorganic iron in the form of ferric ammonium citrate (before autoclaving), hemin (added aseptically after autoclaving, from 1 mg. per ml. filter-sterilized solution of alkaline hematin), or alpha-keto acid (pyruvic acid or alpha-keto glutaric acid, added before autoclaving) as indicated under "Experimental Results." In order to decrease contamination, an amount of malachite green (Coleman and Bell), which was found previously by experiment to have no significant effect on growth of tubercle bacilli on agar media containing 0.5 per cent albumin, was added (1 γ per ml). It should be emphasized that the results obtained were in no way influenced, as determined by comparative studies, by the presence of this concentration of malachite green in media containing 1.5 per cent agar and 0.5 per cent albumin or oleic-acid–albumin complex; at least with the strains studied here.

In some experiments, glycerol (Merck reagent grade) was used at a concentration of 2.0 ml. per liter; under these conditions, Tween® was omitted from all media and, for inocula, Tween®-coated organisms from liquid media were replaced by suspensions of tubercle bacilli cultivated in media containing no Tween®. As previously pointed out (7), glycerol and Tween® 80 are incompatible in media for growth of tubercle bacilli.

Potato tubes (25 mm. x 125 mm.) with metal caps, containing 5 ml. of medium, were used for cultures in liquid media, and Petri dishes (100 mm. x 15 mm.), containing approximately 25 ml. of medium, were used for surface cultures on solid agar.

Preparation of suspensions of dispersed bacterial cells: The method of obtaining well-dispersed suspensions of tubercle bacilli, consisting predominately of single bacterial cells, was identical to that described by Pierce and associates (8) with the following variation:

> Liquid medium cultures of tubercle bacilli were centrifuged and resuspended in distilled water containing 0.2 per cent bovine serum albumin, with or without 0.05 per cent Tween® 80 (as indicated); ground in the Teflon homogenizer; allowed to sediment in the dark for thirty minutes; and the supernate was then filtered under positive pressure through an M-type sintered glass filter. The filtrate, containing well-dispersed bacterial cells, was adjusted to an optical density of 0.125 plus-minus 0.025 on a Beckman spectrophotometer (Model DU) at 0.1 mm. slit opening and 480 millimicrons wave length in a round, soft glass cuvette (diameter, 18 mm.).

Such suspensions of tubercle bacilli (mammalian strains) contain by plate count approximately 5.0×10^7 viable units per ml. Solid agar media planted on a turntable with dilutions of such suspensions were spread evenly with 0.1 or 0.2 ml. of inocula (as indicated), placed in plastic bags (to prevent evaporation), and incubated at 37.5°C. (when incubated under an atmosphere of air containing 5 per cent carbon dioxide in a vacuum-desiccator jar, plastic bags were not employed).

Strains: The following isoniazid-susceptible, parent strains of tubercle bacilli were used:

> *H37Rv* (human type): Obtained through the courtesy of Mr. W. Steenken, Jr., (9) from the Standard Culture Depot, maintained by the National Tuberculosis Association at The Trudeau Laboratory (Saranac, New York).

Vallée (bovine type): Obtained through the courtesy of Dr. R. J. Dubos, Rockefeller Institute, New York City. The parent isoniazid-susceptible virulent culture of this strain is labeled Vallée S.

Pearson (human type): Obtained on primary isolation from a patient at this hospital previous to antituberculous chemotherapy. This virulent strain is labeled Pearson S.

For maintenance of virulence, these strains have customarily been passed through guinea pigs in this laboratory at approximately three-month intervals. The specific studies described here were all made on such recently passed strains. They were preserved in stock culture by passage at weekly intervals through basal medium containing 0.2 per cent glucose, 0.005 gm. ferric ammonium citrate per liter, 0.5 per cent albumin, and 0.05 per cent Tween® 80.

The minimal inhibitory concentration (minimal inhibitory concentration for 99 per cent of the bacterial cells) of isoniazid for these three strains in this medium (with or without Tween® 80) and in the same medium with added hemin or pyruvate is described below:

MINIMAL INHIBITORY CONCENTRATION FOR ISONIAZID (γ PER ML.)

	TWEEN®-ALBUMIN (OR OLEIC-ACID–ALBUMIN) MEDIUM	PLUS 10 γ OF HEMIN PER ML.	PLUS 200 γ OF PYRUVATE PER ML.
H37RvS	0.05	0.15	0.15
Pearson S	0.05	0.15	0.15
Vallée S*	0.2	0.5	0.5

* *See* "Experimental Results" for a description of the difficulty of establishing a minimal inhibitory concentration of isoniazid for this strain.

All isoniazid-resistant mutants of these strains were routinely carried in the same medium, with 10 γ of isoniazid per ml. added.

Catalase tests: Colonies of tubercle bacilli were tested for catalase activity by the method previously described (10). Variations in the final concentration of hydrogen peroxide in the Tween® 80-hydrogen peroxide mixture from 1.5 per cent to 15 per cent had little effect on the results obtained in this test.

EXPERIMENTAL RESULTS

The Effects of Iron, Hemin, Pyruvate, and Manganese on Isolation of Resistant Mutants from H37RvS

The data on the direct isolation of isoniazid-resistant mutants from Tween®-albumin cultures of the native parent susceptible strain of tubercle bacilli, H37RvS, are presented in table 1. The basic medium contained 0.01 gm. of ferric ammonium citrate per liter and 0.5 per cent of oleic-acid–albumin complex and was inoculated with 0.1 ml. of ground, filtered, Tween®-albumin-grown tubercle bacilli at the dilutions indicated. Incubation was in air without added carbon dioxide. It is evident that additional inorganic iron or hemin or pyruvic acid are essential for the isolation of the maximal number of resistant mutants culturable under these conditions and that addition of manganous ions, at the low concentration of 0.1 γ of manganous ions per ml., is potent in inhibiting isolation of resistant mutants unless hemin or pyruvic acid is added to this medium.

TABLE 1

EFFECTS OF IRON, HEMIN, AND PYRUVIC ACID, WITH AND WITHOUT MANGANESE, ON THE PRIMARY ISOLATION OF ISONIAZID-RESISTANT MUTANTS FROM H37RvS ON SOLID AGAR MEDIUM IN ATMOSPHERIC AIR

INOCULA (H37Rv, ISONIAZID-SUSCEPTIBLE)	Basic Medium	Basic Medium Plus:		
		13 γ Fe per Ml.	10 γ Hemin per Ml.	200 γ Pyruvate per Ml.
A. WITHOUT ADDED MANGANESE				
	No Isoniazid			
0.1 ml. of 10^{-6} dilution	100* ++++†	87 ++++	81 ++++	100 ++++
	50 γ Isoniazid per Ml.			
0.1 ml. undiluted	2‡ ++	140 +++	150 ++++	140 ++++
B. WITH 0.5 γ OF MANGANESE PER ML.				
	No Isoniazid			
0.1 ml. of 10^{-6} dilution	130 +++	115 ++++	84 ++++	96 ++++
	50 γ Isoniazid per Ml.			
0.1 ml. undiluted	0	0	140 +++	140 +++

Basic medium: contained 1.5 per cent agar, 0.5 per cent oleic-acid–albumin complex, 0.2 per cent glucose, 0.01 gm. Fe ammonium citrate per liter (1.5 γ Fe per ml).

* Approximate number of colonies after 21 days at 37°C.

† Relative sizes of colonies expressed as: +, microscopic; ++, grossly visible but small; +++ and ++++, large.

‡ Subcultures from these colonies defined as H37RvRy strain (*see* table 2).

Identification of Isoniazid-Resistant Mutants, H37RvRx and H37RvRy

It was almost invariably observed that a few colonies could be obtained on this basic medium, without added iron, hemin, or pyruvate on primary plating of the parent, susceptible H37Rv strain on medium containing 10 to 50 γ of isoniazid per ml. Subculture of such colonies yielded a strain which required the addition of less inorganic iron, hemin, or pyruvate to the basic medium for growth on solid agar. Cultures from such colonies have been labeled H37RvRy and have proved to be distinctly different from the majority of bacterial cells in cultures of the H37RvR strain, as illustrated in table 2. On being plated out on basic 0.5 per cent oleic-acid–albumin (7 H-3) medium without added iron (only 0.01 gm. of ferric ammonium citrate per liter) and without isoniazid but with added manganese, the undissociated R culture has always yielded a variable number of colonies which proved, on subcultivation and testing, to be of the Ry type (figure 1).

Thus, the undissociated R culture consists of two types of mutants, the more fastidious Rx and the less fastidious Ry mutants. The proportion of these two

TABLE 2

GROWTH REQUIREMENTS OF H37RvR AND H37RvRy* STRAINS

INOCULA (0.1 ML. of 10^{-5} DILUTION)	BASIC MEDIUM		BASIC MEDIUM PLUS:					
			15 γ Fe per Ml.		10 γ Hemin per Ml.		200 γ Pyruvate per Ml.	
	No Isoniazid							
	No Mn	0.1 γ Mn per Ml.	No Mn	0.1 γ Mn per Ml.	No Mn	0.1 γ Mn per Ml.	No Mn	0.1 γ Mn per Ml.
H37RvR	90	8	80	90	70	90	90	90
(x + y)	+++	++	++++	+++	++++	++++	++++	++++
H37RvRy*	70	70	70	80	70	70	70	70
	++++	++++	++++	++++	++++	++++	++++	++++
	50 γ Isoniazid per Ml.							
H37RvR	90	2	90	25	80	90	90	90
(x + y)	+++	++	++++	++	++++	++++	++++	++++
H37RvRy	70	70	70	70	80	70	70	70
	+++	+++	++++	++++	++++	++++	++++	++++

Same basic medium as described in table 1.

Both strains maintained by subcultures in Tween®-albumin medium containing 10 γ of isoniazid per ml.

N.B. *See* table 1 for legends.

Inocula: from Tween®-albumin medium containing 0.5 per cent albumin and 10 γ of isoniazid per ml.

* H37RvRy derived from H37RvS as described in table 1.

mutants in the undissociated R culture varies somewhat (100 Rx:1 Ry to 10 Rx:1 Ry) from experiment to experiment. It is not yet known whether the Ry strain may dissociate to give off Rx-type mutants. It should be noted that both the Rx-type and the Ry-type mutants of the H37Rv strain thus far encountered by plating out on media containing more than 0.25 γ of isoniazid per ml. have proved to be "catalase-negative" (10).

Because pyruvic acid (11) and hemin under certain conditions, especially with manganese (12), may be antagonists of isoniazid, additional studies were made to determine the possible antagonistic activities of inorganic iron, hemin, and pyruvate under the conditions used in these experiments. None of these substances was shown to have sufficient antagonistic activity to account for the differential growth requirements of the isoniazid-susceptible parent strain and the two isoniazid-resistant mutants. In similar studies with liquid media, to be reported elsewhere (13), it was observed that the presence of isoniazid simply increases somewhat the special growth requirements of the isoniazid-resistant mutants for inorganic iron, which has no intrinsic antagonistic activity toward isoniazid. The presence of isoniazid likewise increases the need for certain organic-iron-compounds or for alpha-keto acids, most clearly in the presence of added traces of manganese.

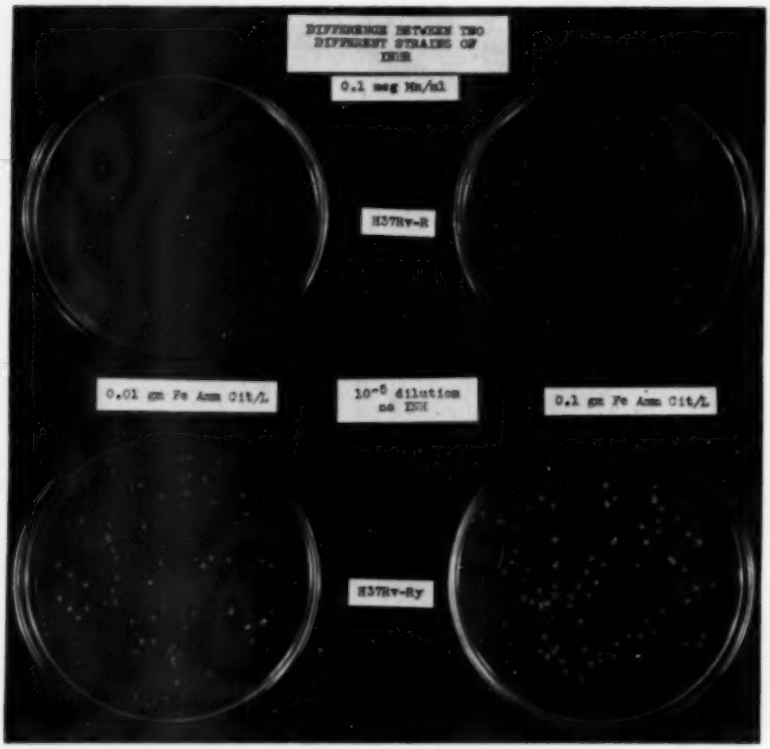

FIG. 1. Comparative growth of the undissociated H37RvR culture, containing both the more fastidious Rx and the less fastidious Ry mutants, and the pure culture of the H37RvRy mutant on the basic agar medium (containing 0.2 per cent glucose, 0.2 per cent glycerine, and 0.5 per cent oleic-acid-albumin complex) with relatively low and with higher concentrations of inorganic iron. The evident differences were brought out by the addition of 0.1 γ of manganese per ml. of medium. This amount of manganese is insufficient to inhibit a good growth of the Ry mutant on this medium, whereas the Rx mutant, constituting the great majority of the population of the R strain, was unable to multiply. Well-dispersed bacterial cells were used as inocula in this experiment.

A comparison of the data in tables 1 and 2 shows that the H37RvR strain, once isolated and subcultivated through a few transfers in basic medium with Tween®- albumin, containing 10 γ of isoniazid per ml., manifests a lesser iron requirement than is necessary for good growth during the process of primary isolation. This phenomenon has been repeatedly confirmed in additional experiments with the H37Rv and with other mammalian strains.

Comparative Studies on Different Isoniazid-Susceptible Strains

Results essentially similar to those obtained with the H37RvS strain have been obtained with two other strains, Vallée S and Pearson S. However, strain

TABLE 3

PRIMARY ISOLATION OF ISONIAZID-RESISTANT MUTANTS FROM THREE DIFFERENT PARENT, ISONIAZID-SUSCEPTIBLE, VIRULENT STRAINS OF TUBERCLE BACILLI

INOCULA APPROXIMATELY 10^7 BACTERIAL CELLS	BASIC MEDIUM* PLUS: CONCENTRATION OF ISONIAZID (γ per Ml.)											
	10γ Hemin per Ml.						200 γ Pyruvate per Ml.					
	0.25	0.5	1.0	5	20	50	0.25	0.5	1.0	5	20	50
H37RvS	200† ++++	200 ++++	200 ++++	200 ++++	200 ++++	200 ++++	200 ++++	200 ++++	200 ++++	200 ++++	200 ++++	200 ++++
Pearson S	166‡ +++	80‡ +++	80 +++	26 +++	16 +++	19 +++	200‡ +++	100 +++	22 +++	21 +++	14 +++	14 +++
Vallée S	innumerable ++++	>400 ++++	>400 ++++	75 ++++	75 ++++	75 ++++	>400 ++++	100 ++++	75 ++++	75 ++++	75 ++++	75 ++++

* Basic medium: 0.5 per cent oleic-acid–albumin medium with 0.2 per cent glucose, 0.05 gm. Fe ammonium citrate per liter.

† Recorded colony counts above 50 are only approximate; growth after 21 days.

‡ A few catalase-positive colonies (8) were detected in populations on these plates only, when catalase tests were performed on all plates at the termination of the experiment.

variations in the incidence of isoniazid-resistant mutants at different concentrations of isoniazid in the culture medium are clearly demonstrated in table 3. In performing this experiment, a basic medium containing 0.05 gm. of ferric ammonium citrate per liter, 0.5 per cent oleic-acid–albumin, and 0.2 per cent glucose was used. Because of the relatively low incidence of highly resistant mutants in Pearson S cultures, 0.2 ml. instead of 0.1 ml. inocula of undiluted cultures from Tween®-albumin medium were employed. Media containing either added hemin or pyruvic acid were used in order to permit the isolation of as many resistant mutants as possible.

In a previous communication (14) it was reported that, with the Vallée and R1Rv strains, the incidence of isoniazid-resistant mutants was approximately the same over a wide range of concentrations of isoniazid above the minimal inhibitory concentration for the particular strain. It has subsequently been observed, however, that this is not necessarily true with populations of tubercle bacilli on primary isolation from the sputum of isoniazid-treated patients (6). The decreasing incidence of isoniazid-resistant mutants from drug-susceptible populations of the more recently isolated Pearson strain (*see* table 3) illustrates that there are significant strain differences in this respect.

It is also noteworthy that the results with the Vallée strain differ in this particular experiment from the other two strains in the high incidence of colonies which appear to be capable of growing at low concentrations of isoniazid. These concentrations do have inhibitory activity against most of the bacteria in the parent drug-susceptible populations. The colony counts given in table 3 as >400 were only a small fraction of the original inoculum of approximately 10^7

bacterial cells, even in medium containing 0.5 γ of isoniazid per ml. with added hemin.

With regard to this interesting phenomenon, it can only be stated at present that the majority of the small colonies from this strain which appear on media containing less than 1.0 γ of isoniazid per ml. are clearly different from those few mutants which, on primary isolation from isoniazid-susceptible populations, are resistant to high as well as to low concentrations of the drug. Subcultures of these small colonies to Tween®-albumin liquid medium containing no isoniazid and plating out of such cultures, simultaneously with the parent drug-susceptible strain, on 0, 0.05, 0.075, 0.1, and 0.15 γ of isoniazid per ml. revealed that they were not resistant mutants and are catalase-positive. For obvious reasons they were called "pseudo-mutants."

The only explanation available at the present time is that these organisms, present in the unavoidably heterogeneous parent drug-susceptible culture, were phenotypically, not genotypically, different from the majority of the bacteria in the population planted; that they were metabolizing at a much slower rate and, therefore, not immediately sterilized by isoniazid, which slowly lost activity to concentrations at which these organisms could then multiply. The small size of this type of colony on the original plates is consistent with this hypothesis. Also, consistent with this hypothesis is the observation that such colonies appeared most commonly on agar plates which contained either hemin, an agent which catalyzes the oxidation of isoniazid extracellularly (15), or pyruvate, a substance which may have a similar effect on isoniazid (11).

Most important for the previously described (1) attenuation of most isoniazid-resistant (1 γ per ml.) mutants of tubercle bacilli for guinea pigs is the observation that catalase-positive, isoniazid-resistant mutants appear on primary isolation from parent drug-susceptible populations only in media containing minimal inhibitory concentrations of isoniazid and that, even under these conditions, they are fewer in numbers than are the more resistant, catalase-negative mutants. This phenomenon is clearly evident in table 3. Of the three strains described, only from the recently isolated Pearson S strain did catalase-positive, resistant mutants emerge and then only in the lower concentrations of isoniazid.

Studies Under an Atmosphere of Air Containing 5 Per Cent Carbon Dioxide

The experiments described above were carried out by incubating cultures in ordinary atmospheric air. They were repeated under an atmosphere of air containing 5 per cent carbon dioxide, and the results obtained were essentially the same, except that growth of all strains[3] was more rapid and the colonies were, therefore, much larger after 21 days of incubation. In addition, under these conditions the "pseudo-mutant" colonies described above either did not appear or appeared in very much smaller numbers.

[3] Cultures of the stock laboratory strains, H37Rv and Vallée, gave poor growth under 5 per cent carbon dioxide unless they were recently passed through a susceptible host. The possible significance of this phenomenon will be described elsewhere in detail (16).

DISCUSSION

The application of quantitative methods and the use of flat-surfaced agar media containing bovine serum albumin in Petri dishes has permitted the detection of three different types of isoniazid-resistant mutants of virulent tubercle bacilli under artificial conditions *in vitro*. The use of a well-defined basic medium was essential to the discovery that two of these mutants have quantitatively different growth requirements for hemin, or for alpha-keto acids, but that both of them are more fastidious in their requirements than the parent drug-susceptible strains. In particular, these two mutants require hemin, an alpha-keto acid, or higher concentrations of inorganic iron than are required by their parent strains; and the addition of mere traces of manganous ions, of the order of one part per 10 million parts of medium, increases their requirements for these additional substances. A more detailed analysis of these growth requirements will be presented elsewhere (13). Suffice it to point out here that the addition of 15 γ of iron per ml. in the form of ferric ammonium citrate to the basic medium employed is sufficient to permit good growth of these catalase-deficient strain. Moreover, the addition of iron, under these conditions, in contrast to the addition of hemin (12) or pyruvate (11), does not antagonize isoniazid as an antimicrobial agent against tubercle bacilli.

Inasmuch as catalase-positive, isoniazid-resistant mutants of tubercle bacilli have been detected in the sputum of tuberculous patients (6), it is of some interest that such mutants may be isolated from drug-susceptible populations of at least one freshly isolated strain under artificial conditions *in vitro* but only in the presence of low concentrations of isoniazid.

Quantitative differences in the incidence of drug-resistant mutants from different strains of otherwise very similar microorganisms is not novel. As applied to the microbiology of tuberculosis, however, the data reported here indicate that, so far as the emergence of drug-resistant populations is concerned, the properties of the individual strain of tubercle bacilli harbored by a particular patient before initiation of isoniazid therapy may play as important a role in determining the response to chemotherapy as the anatomic distribution or character of the lesions or other factors related primarily to the response of the host.

SUMMARY

Three different types of isoniazid-resistant mutants have been identified in parent drug-susceptible populations of three strains of virulent tubercle bacilli. Two of these mutants are both markedly deficient in catalase activity. Nevertheless, they are distinguishable by quantitative differences in their growth requirements for hemin, alpha-keto acids or, under the conditions described here, inorganic iron, particularly in agar medium containing added manganous ions. The third mutant type is not so highly deficient in catalase activity. Mutants of this type make their appearance in relatively small numbers only on media containing low concentrations of isoniazid. On direct isolation from the parent drug-susceptible population, they appear to be only weakly resistant to isoniazid.

Important differences have been observed between different strains of tubercle bacilli. The numbers of mutants resistant to any one concentration of isoniazid may vary among different strains. Catalase-positive, resistant mutants have been identified in only one of three strains studied under artificial conditions *in vitro*.

SUMARIO

Estudios de la Isoniacida y los Bacilos Tuberculosos. I. El Aislamiento de Mutantes Isoniacidorresistentes in Vitro

En las poblaciones paternosusceptibles de tres cepas de bacilos tuberculosos virulentos, se han identificado tres distintas formas de mutantes isoniacidorresistentes. Dos de esas mutantes son a la vez notablemente deficientes en su actividad ante la catalasa, pero cabe distinguirlas por diferencias cuantitativas en sus requisitos de desarrollo para la hemina, los ácidos alfa-queto o, en las condiciones aquí descritas, para el hierro inorgánico, en particular en el medio de agar que contiene yones manganosos agregados. La tercera forma de mutante no es tan deficiente en su actividad catalásica. Mutantes de esa forma aparecen en cantidades relativamente pequeñas únicamente en medios que contienen concentraciones bajas de isoniacida: al aislarlas directamente de la población paterna sensible, parecen ser sólo débilmente resistentes a la isoniacida.

Se han observado importantes diferencias entre distintas cepas de bacilos tuberculosos: los números de mutantes resistentes a cualquiera concentración de isoniacida pueden variar entre distintas cepas; se han identificado mutantes resistentes, catalasa-positivas solamente en una de tres cepas estudiadas en condiciones artificiales *in vitro*.

RESUME

Etudes sur l'isoniazide et le bacille tuberculeux: 1. L'isolement in vitro des mutants isoniazide-résistants

Trois types différents de mutants isoniazide-résistants ont été identifiés dans les populations de trois souches de bacilles virulents issues d'une souche initiale sensible. Deux de ces mutants sont nettement déficients dans leur activité catalasique, mais à l'égard du développement, ils se distinguent par des différences quantitatives dans leurs besoins en hémine, acides alpha-céto ou, dans les conditions de l'expérience, en fer inorganique et, en particulier, dans le milieu agar avec ions manganèse supplémentaires. Le troisième type mutant ne présente pas une déficience aussi marquée de l'activité catalasique. Les mutants de ce type ne font leur apparition qu'en nombre relativement réduit dans les milieux renfermant de faibles concentrations d'isoniazide; lorsqu'ils sont obtenus par isolement direct à partir de la souche initiale, population sensible, leur résistance à l'isoniazide est faible.

Des différences importantes ont été observées entre les différentes souches de bacilles tuberculeux; le nombre de mutants résistants à l'isoniazide aux différentes concentrations peut varier parmi les différentes souches; les mutants résistants catalase-positifs, n'ont été isolés que dans une seule des trois souches étudiées dans des conditions artificielles *in vitro*.

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EFFECT OF ISONIAZID UPON THE EMOTIONS AND THE SERIAL ELECTROENCEPHALOGRAMS OF TUBERCULOUS PATIENTS[1, 2]

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INTRODUCTION

Among the antimicrobial agents presently available, isoniazid is one of the most effective. Its action in meningeal and miliary tuberculosis is unparalleled (1); in pulmonary and other common forms of tuberculosis it seems to match the results achieved by the time-tested combination of streptomycin and para-aminosalicylic acid (PAS) (2). The action is even more marked when the isoniazid is given concurrently with one of the other effective antimicrobial agents (3). Although there is little doubt today of the remarkable efficacy of isoniazid in tuberculosis, there is considerable divergence of opinion concerning the drug's toxicity. An authoritative report (4) on isoniazid listed such side effects as muscle twitchings, hyperreflexia, restlessness, insomnia, headache, and occasionally convulsions. Also encountered are a "mild euphoria and excitability" (5). "Subtle changes in personality" are said to be probably "the commonest manifestation" of the drug's toxicity, but "very difficult to evaluate" (4).

In psychotic tuberculous patients, the administration of isoniazid was found to be followed by an improvement in mental status (6). Anderson, on the other hand, suggested that isoniazid be withheld in tuberculous patients with organic disease of the central nervous system, or with abnormal signs or history, for fear of aggravating the mental status (7). This view was supported by the work of Ferrara and Peterson (8), whose experience led them to the conclusion that isoniazid was not an innocuous drug. In another series of similar patients, however, it was found that those whose psychoses were definitely related to their tuberculosis were benefited by isoniazid while, in patients whose psychoses antedated their tuberculosis, isoniazid appeared to have no effect on the mental status (9). Episodes of psychoses have been reported also in apparently normal tuberculous patients who were treated with isoniazid (6). Tessing-Ericson reported 3 cases in which alarming psychoses developed after prolonged treatment with isoniazid (10). One of the patients received only 300 mg. daily for 6 months and developed increasing paranoid ideas and hallucinations. In animal experiments, it was found that very large doses of isoniazid produced widespread toxic effects, including involvement of the central nervous system, evidenced by such symptoms as "apprehension, anxiety, and convulsions" (11). Even with more nearly therapeutic doses (10 mg. per kg. per day) ataxia, tremors, and con-

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[2] Presented, in part, before the Thirteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, St. Louis, Missouri, February 8, 1954.

vulsions were produced after ten weeks of administration (12). With similar doses of 10 to 20 mg. per kg., isoniazid was found to cause bursts of high-amplitude spikes in the electroencephalograms of anesthetized dogs, resembling the effect of picrotoxin (13).

Observations such as the above have led the present writers to study the psychologic status of tuberculous patients, presumably normal mentally, who were being treated with therapeutic doses of isoniazid. Serial electroencephalographic records of the patients were likewise studied.

PLAN OF INVESTIGATION

At the Veterans Administration Medical Teaching Group (Kennedy) Hospital, during the fourteen months beginning June 15, 1952, 71 patients were treated with isoniazid. Of these, 52 were studied with serial electroencephalograms. The 19 patients excluded consisted of 2 who had received isoniazid elsewhere, 14 who were considered too ill for these studies, and 3 who refused to cooperate. Psychologic tests were administered also to the first 40 of the 52 patients in the study. Four patients received 150 mg. of isoniazid daily; all others received 300 mg. divided into three doses daily. Except for the first 17 patients who received isoniazid alone, all received additional chemotherapy (usually PAS or streptomycin) throughout the period of study. The group consisted of 32 white males, 17 Negro males, and 3 white females. The patients ranged in age from eighteen to sixty-four years, with a mean of forty-two years. Six patients had minimal pulmonary tuberculosis, 15 moderately advanced, and 29 had far advanced disease. One patient had tuberculous meningitis, and another had tuberculosis of the cervical lymph nodes.

METHOD AND PROCEDURE

A few days prior to the first dose of isoniazid, an initial brain wave tracing was obtained, and the following psychologic tests were made: the Rorschach, the Bender-Gestalt, and the Depression Scale of the Minnesota Multiphasic Personality Inventory (MMPI).

The Rorschach was chosen to study the effects of the drug on the patient's personality structure and emotion, as inferred from his interpretation of unstructured ink-blots. The Bender-Gestalt, a test in which the patients reproduce geometric designs, was used to study the effects on visuomotor coordination and mood and to detect organic brain involvement. The Depression Scale of the MMPI, a true-false type of questionnaire, was used to determine the variation in the number of answers indicative of depression. In this test, the patient would answer true or false to questions such as, "I am happy most of the time," or "I usually feel that life is worth while," et cetera. These same psychologic tests were readministered eight to ten weeks following the initiation of treatment with isoniazid.

Routine waking electroencephalograms were obtained at monthly intervals initially, but then, as no significant changes were noted, the tracings were obtained at intervals of two to three months. The number of electroencephalograms varied from two to nine per patient, with an average of four and a total of 210 for the 52 patients. All patients were observed clinically by their own ward physicians for abnormal behavior and for the appearance of abnormal neurologic findings.

RESULTS

Of the 52 patients on whom electroencephalographic studies were made, 10 had tracings prior to treatment which were considered outside of normal limits

TABLE 1

ELECTROENCEPHALOGRAPHIC FINDINGS

INITIAL EEG	NUMBER OF PATIENTS	NUMBER OF TRACINGS	CHANGES DURING TREATMENT	
			Toward Normal	Toward Abnormal
Normal	42	158	—	1
Abnormal	10	51	1	1 (more abnormal)
Total	52	209	1	2

(table 1). One of these patients, with a slow record prior to treatment, showed consistently normal ones during treatment. However, another patient with a slow record prior to treatment gave electroencephalographic tracings which became progressively more abnormal during treatment. He was followed for fourteen months, and two tracings obtained during the three months following the cessation of isoniazid therapy were more abnormal than those obtained during the period when he was on therapy. In the 42 patients whose preliminary tracings were essentially normal, there was a significant change in the record of only one person. After two months of isoniazid therapy, the brain waves in this patient became abnormally slow, and since then they have become progressively more abnormal.

Thus far, electroencephalographic records have been obtained after the conclusion of the isoniazid therapy in 17 patients. In only one (the patient already described) was there a change in the tracing after termination of therapy. Thus, in the 52 patients studied, only 3 showed any changes in the character of the electroencephalographic tracings. In one, they changed from normal to abnormal, in another from abnormal to normal, and in the third from abnormal to more abnormal. From these data, no definite statement can be made, but it appears that the effect of isoniazid on the brain waves of the great majority of the patients studied was negligible.

Each of the 40 patients who received psychologic testing was asked to give his opinion of how he felt eight to ten weeks after the start of therapy, taking into account "sense of well-being," "spirits," and "mood" (table 2). Thirty-three rated themselves as improved in various degrees. Only 7 considered themselves unimproved, and not one patient reported himself as worse. Nonetheless, the

TABLE 2

PATIENTS' SUBJECTIVE RATINGS OF IMPROVEMENT AFTER EIGHT TO TEN WEEKS OF ISONIAZID THERAPY

WORSE	NO CHANGE	SOME IMPROVEMENT	MUCH IMPROVEMENT	VERY MUCH IMPROVEMENT
0	7	10	10	13

(Patients' own selection of category)
Total number of patients—40

TABLE 3

COMPARISON OF TEST-RETEST RESULTS OF NINE PATIENTS, ORIGINALLY OBVIOUSLY DEVIANT (Bender-Gestalt)

PATIENT	TEST SCORE	RETEST SCORE	CHANGE*
1. F. W.	71	78	+7
2. P. K.	77	69	−8
3. J. C.	73	104	+31
4. W. H.	67	96	+29
5. A. S.	41	60	+19
6. S. H.	80	71	−9
7. D. P.	66	44	−22
8. E. A.	45	44	−1
9. J. K.	51	55	+4

* + = Amount of change in direction of abnormal
− = Amount of change in direction of normal

psychologic tests failed to corroborate these subjective ratings. The Rorschach tests, in general, failed to show any significant changes between test and retest performances except in the case of one patient whose retest record indicated even greater constriction and depression.

The Bender-Gestalt test showed no evidence of any definite effects on the central nervous system. It, too, failed to show greater expansiveness, consistent with a more euphoric mood, on the second administration of the test. Thirty-one of the patients showed essentially no change, 3 showed a greater expansiveness, and 6 a lesser expansiveness. The retest results were of interest in 9 patients of the original 40, who in their pretreatment tests showed obviously deviant figure reproductions. A comparison of the retests with the original test performances, using the Pascal-Suttell (14) scoring system, revealed that 4 of the 9 patients showed higher scores (more "abnormal"), 3 showed lower scores (less "abnormal"), while 2 patients had records essentially unchanged (table 3).

The scores on the Depression Scale of the MMPI also showed no significant test–retest changes. The mean difference was only 1.53, which is not statistically significant, although whatever difference there is does lie in the direction of decreased depression.

Clinically, too, no significant change was noted in the behavior or neurologic findings of any of the 52 patients, except one. A brief summary of this exception is given because, with either a less thorough study or a less fortunate follow-up, an erroneous conclusion might readily have been drawn.

F. W., a 35-year-old Negro with far advanced pulmonary tuberculosis and bilateral extensive cavitation, had been under treatment for more than two years with bed rest and various chemotherapeutic agents and had reached stabilization of disease without reversal of infectiousness. On September 3, 1952, 100 mg. of isoniazid three times daily was added to his regimen of streptomycin, 1 gm. twice weekly, and PAS, 12 gm. daily. By January, 1953, three months later, the patient's former cooperative and agreeable behavior was noted by the ward physician to be changing to an agressive, restless, and suspicious attitude. The neurologic examination was normal. A psychiatric consultant found strong evidence of a

paranoid development. The patient's attitude did not improve, and he left against medical advice on March 1, 1953. When he returned to the hospital on October 6, 1953, he stated that at home without medications he had become "less nervous" in approximately two weeks. His behavior was again found to be pleasant and cooperative. However, despite the reinstitution of isoniazid therapy, which has been maintained for more than four months, the patient has shown no regression toward paranoid behavior.

A brain wave tracing on this patient taken prior to the initial therapy had shown low amplitude, mixed slow and fast activity which was nonspecific but suggestive of a diffuse organic brain involvement. Three tracings taken during therapy showed no change. Another taken when the patient returned to the hospital in October, 1953, again showed no change. In the psychologic testing of this patient prior to isoniazid therapy, both the Bender-Gestalt and the Rorschach tests indicated organic brain damage. Two months later, just before the patient's behavior was noticeably paranoid, the tests were repeated, and the Bender-Gestalt was found to have become more abnormal while the Rorschach remained essentially the same, still indicative of organic brain damage.

DISCUSSION

The relation of the isoniazid to the single psychotic episode noted is difficult to evaluate. It is believed, however, that the evidence available does not definitely indict the isoniazid as the cause. From the time the present study was instituted, 4 other patients, not in the study, developed psychotic episodes. Yet, although two-thirds of the patients receive isoniazid, 2 of the 4 patients who developed psychoses had not been on isoniazid therapy. Persons with psychotic tendencies who develop tuberculosis do not necessarily require isoniazid to become frankly psychotic.

The objective findings obtained from the serial electroencephalographic tracings and from the battery of psychologic tests failed to demonstrate any definite effect of isoniazid upon the central nervous system or upon the emotions of the patients tested. The feeling of well-being expressed by the patients probably represented perception of the beneficial effect of isoniazid upon the tuberculosis rather than an effect of the drug per se upon the emotional state or the central nervous system.

The present material is insufficient to prove definitely whether isoniazid is likely to produce frank psychotic episodes in persons predisposed to psychoses. In the one patient in whom this seemed to happen, an attempt to reproduce the psychosis with isoniazid failed. The present results do show that it is the exception rather than the rule for frank psychosis to occur in presumably predisposed patients who are treated with therapeutic doses of isoniazid. Certainly, there seems to be no reason to withhold this drug from such patients when it is indicated. On the other hand, the present study failed to support the view that isoniazid in itself tends to improve the mental status of such patients.

SUMMARY

Fifty-two unselected patients were studied with serial electroencephalograms while being treated with 150 to 300 mg. of isoniazid daily. The first 40 patients were studied also with a battery of psychologic tests.

Only 3 patients showed any electroencephalographic changes, one from normal to abnormal, one from abnormal to more abnormal, and one from abnormal to normal. From these results, no definite effect of the isoniazid on the brain tracings could be discovered.

While the great majority of patients claimed improvement in "well-being" and "spirits" and none felt worse, the objective tests failed to show any definite change in the emotions of these patients.

In 9 patients who, from their preliminary psychologic tests, might be presumed to be predisposed to psychosis, 4 showed some worsening, 3 some improvement, and 2 showed no change in their mental status.

Clinically, one patient became psychotic. This disappeared on cessation of isoniazid therapy, but did not reappear on the resumption of the same therapy.

No definite effect could be noted from isoniazid in a dosage of 150 to 300 mg. daily on the emotions or on the central nervous system of the great majority of patients studied.

There seems little reason to withhold isoniazid when it is indicated because of the emotional or the mental status of a tuberculous patient.

SUMARIO

Efecto de la Isoniacida sobre las Emociones y los Electroencefalogramas Seriados de los Tuberculosos

Cincuenta y dos enfermos tomados al azar fueron estudiados con encefalogramas seriados mientras se les trataba con 150 a 300 mg. de isoniacida diariamente. Los primeros 40 enfermos fueron además estudiados con una serie de pruebas psicológicas.

No pasaron de 3 los enfermos que revelaron la menor alteración electroencefalográfica: uno de normal a anormal, uno de anormal a más anormal y uno de anormal a normal. A juzgar por esos resultados, no pudo descubrirse ningún efecto bien definido de la isoniacida sobre los trazados cerebrales.

Aunque la inmensa mayoría de los sujetos pretendieron haber mejorado en "bienestar" y "ánimo" y ninguno se sentía peor, las pruebas objetivas no revelaron el menor cambio preciso en las emociones de esos enfermos.

De 9 enfermos en quienes, a base de las pruebas psicológicas preliminares, cabía presumir predisposición a psicois, 4 mostraron algún empeoramiento, 3 alguna mejoría, en tanto que 2 no mostraron alteración.

Clínicamente, un enfermo se volvió psicótico. Esto desapareció al cesar la isoniacidoterapia, pero no reapareció al reanudarse ésta.

RESUME

Action de l'isoniazide sur les émotions et les électro-encéphalogrammes en série chez le tuberculeux

Cinquante-deux malades non-sélectionnés ont été étudiés à l'aide d'électroencéphalogrammes en série au cours d'un traitement par l'isoniazide à la dose de 150 à 300 mg par jour. Les 40 premiers malades ont été également étudiés à l'aide d'une "batterie" de tests psychologiques.

Trois malades seulement ont présenté une altération du tracé électroencéphalographique, un de normal à anormal, un d'anormal à plus anormal et un d'anormal à normal. Ces résultats ne permettent pas de conclure à une action définie de l'isoniazide sur les tracés électroencéphalographiques.

Bien que la grande majorité des malades aient signalé une grande amélioration de leur

état physique et de leur "moral", et qu'aucun d'entre eux ne se soit senti plus mal, les tests objectifs n'ont pas permis d'observer de modification émotionnelle définie chez ces malades.

Parmi 9 malades que des tests psychologiques préliminaires désignaient comme prédisposés aux psychoses: 4 ont présenté un certain degré d'aggravation, 3 une certaine amélioration et 2 aucun changement.

Cliniquement, 1 malade est devenu psychotique. Cet état a disparu quand l'isoniazide-thérapie a été interrompue et il ne s'est plus reproduit quand cette médication a été reprise.

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A SCALE FOR RATING THE WARD BEHAVIOR OF HOSPITALIZED TUBERCULOUS PATIENTS[1]

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INTRODUCTION

Ward behavior among hospitalized tuberculous patients is a matter of considerable importance to everyone concerned with the treatment of tuberculosis. From the medical point of view, the cooperative behavior of the patient constitutes an essential part of his therapeutic regimen. Strict adherence to bed-rest schedule is regarded as a *sine qua non* for successful treatment. Emotional and mental relaxation, as well as physical rest, are also considered desirable and perhaps indispensable for optimal results (1, 2). From the administrative point of view, the degree to which patients accept the rules and requirements of the hospital or sanatorium is closely tied to such problems as the maintenance of discipline and morale and the effective fulfillment of hospital policies.

Unfortunately, the stressful nature of prolonged hospitalization is such as to make it difficult for many tuberculous patients to adhere to the mode of life prescribed by the medical and administrative staff. Patients often resent the prohibitions against physical activity, the absence of privacy, the regimented community life, and the enforced separation from accustomed activities. Patients commonly respond by complaining excessively, violating their bed-rest classification, manifesting hostility toward authority, and otherwise displaying "unreasonable" forms of ward behavior. For many patients, tuberculosis carries a sense of social stigma and a feeling of being ostracized. It means also a forced childlike dependence on the ward personnel—a dependence which in most cases runs directly counter to everything heretofore expected of the patient as an adult. It is not difficult to see why, in the face of these stressful circumstances, patients often react with restlessness, depression, anxiety, resentment, apathy, and other signs of emotional disturbance. It is equally apparent that such reactions do not enhance the possibility of obtaining the mature cooperation which is medically so desirable. It is quite probable that failure of the hospital personnel to deal adequately with these patient problems is an important contributing factor to an excessive need for disciplinary action and to a high incidence of irregular discharge (3, 4, 5).

The first task facing anyone interested in coping with the problems of ward behavior is to obtain an accurate picture of how the patient actually does behave. How well does he adhere to bed-rest schedules? To what extent does he conform to hospital regulations? Does he get along well with the doctors, the nurses, and with his fellow patients, et cetera? The purpose of this paper is to

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describe the development of an objective scale for assessing these and other aspects of ward behavior among tuberculous patients.

MATERIALS AND METHODS

In the course of developing several lines of research at this hospital, the problem of obtaining an accurate measure of patients' ward behavior presented itself. Specifically, it was believed that a knowledge of the extent of a patient's misbehavior on the ward would provide us with clues to the probability of his leaving irregularly. It was also thought to be of interest to attempt to discover whether the patient's ward behavior had any meaningful relationship to his medical progress.

The customary way of getting information about a patient's behavior is to ask a staff member, usually a ward nurse, for her impression of the patient. There was sufficient evidence from past experience, however, to indicate that this method did not always give reliable information. It was observed at staff conferences, for example, that the same patient might be judged quite differently by various members of the staff. Two nurses handling the same patient might see him in opposite ways, one regarding him as a model patient, whereas the other might believe that he wandered around the ward too much, complained constantly, and was a bad influence on the other patients. Similarly, the impression of the physician might differ markedly from that of the social worker or other staff members. Sometimes these divergent views tended to supplement each other, resulting in a fuller picture of the patient. At other times the differing views were so contradictory as to make it probable that the individual biases of staff members, their particular likes and dislikes for the patient, and other such subjective factors were responsible for the discrepancies between judgments about the patient. For this reason it was believed to be necessary to develop a more objective and reliable method for getting this information.

Two methods suggested themselves. A qualified staff member, such as a head nurse, could be asked to give her over-all impression of a patient's behavior as compared with the behavior of other patients. For example, if the question was one of rating a patient with regard to his cooperativeness on the ward, the nurse would be asked to rate him as belonging to the 25 per cent of her most cooperative patients, to the 25 per cent of her least cooperative patients, or to the 50 per cent of her middle or average group of patients. A second approach would be to have the staff member rate each patient on a number of specific actions which commonly occur among patients. The presence and frequency of these specific acts in a given patient would then provide a criterion for comparing his ward behavior with that of other patients.

It was decided to test both approaches and to compare their merits. The general plan was to have a ward head nurse and one of her ward nurses independently rate a sample of patients from their ward using both methods and then to compare the inter-rater reliabilities of the two methods.

Two wards participated in the study, with 16 patients from one ward and 20 patients from the second ward comprising the total sample of 36 subjects.

The first method was represented by two questions:

1. How would you rate this patient as to general cooperativeness?
   ——Better than average (Top 25 per cent of patients on your ward)
   ——Average (Middle 50 per cent of patients on your ward)
   ——Poorer than average (Poorest 25 per cent of patients on your ward)

2. How would you rate this patient as a bed-rest patient?
   ——Better than average (Top 25 per cent of patients on your ward)
   ——Average (Middle 50 per cent of patients on your ward)
   ——Poorer than average (Poorest 25 per cent of patients on your ward)

The second approach was represented by a ward behavior scale, which was constructed in the following manner: The nurse in charge of patient education and the head nurses of four bed wards submitted lists of what they believed to be the most common forms of patient behavior deemed undesirable from the staff point of view. The lists were compared, and those forms of behavior which appeared most frequently were chosen for inclusion in a ward behavior scale. Fifty items were finally selected which could be roughly categorized into five general areas of undesirable behavior: (*1*) violation of bed-rest classification (items 1–4), (*2*) disregard for hospital rules (items 5–12), (*3*) resistance to medical requirements other than bed rest (items 13–19), (*4*) complaining and demanding behavior (items 20–36), and (*5*) unsatisfactory relations with staff members and fellow patients (items 37–50). These items were incorporated into a ward behavior rating scale, which is reproduced below:

WARD BEHAVIOR SCALE FOR TUBERCULOUS PATIENTS

Patient's Name _____ Reg. No. _____ Ward _____ Class _____ Date _____
How long have you known the patient? _____ months; _____ weeks
Date of admission _____ Admission-Rating interval _____

INSTRUCTIONS TO OBSERVER: The purpose of this scale is to help you describe the ward behavior of the patient named above. The scale consists of a number of statements which describe some of the ways patients act on the ward. You are to mark each statement as *occurring frequently*, *occurring occasionally*, or *never occurring* with respect to the above-named patient. If the activity referred to occurs frequently, circle *F*. If the activity occurs occasionally, circle *O*. If it never occurs, circle *N*.

Be sure to rate each statement. Don't leave any out.

1.	F	O	N	The patient does not observe his bed rest schedule.
2.	F	O	N	The patient wanders around out of his room when he is not supposed to.
3.	F	O	N	The patient doesn't follow the rest period schedule.
4.	F	O	N	The patient abuses his medical classification by going to the bathroom excessively.
5.	F	O	N	The patient is not around when wanted for strep shots, X-ray appointments, etc.
6.	F	O	N	The patient congregates in the bathroom with other patients.
7.	F	O	N	The patient visits other patients against rules.
8.	F	O	N	The patient is suspected of drinking on the ward.
9.	F	O	N	The patient is suspected of gambling on the ward.
10.	F	O	N	The patient does not cooperate with early morning routines.
11.	F	O	N	The patient misuses the telephone privilege.
12.	F	O	N	The patient uses foul language.
13.	F	O	N	The patient does not take care of his personal hygiene.
14.	F	O	N	The patient spits carelessly.
15.	F	O	N	The patient doesn't cover his coughs.
16.	F	O	N	The patient refuses his medication.
17.	F	O	N	The patient threatens to leave AMA.
18.	F	O	N	The patient sets his own time limit for his stay in the hospital.

19. F O N The patient is suspicious of medication given to him.
20. F O N The patient complains about the food.
21. F O N The patient complains about the entertainment program.
22. F O N The patient complains about the occupational therapy facilities.
23. F O N The patient complains about the visitor situation.
24. F O N The patient complains about the pass situation.
25. F O N The patient complains about the lack of privacy.
26. F O N The patient complains about being ill.
27. F O N The patient complains about favoritism to other patients.
28. F O N The patient complains about many things unconstructively.
29. F O N The patient expresses resentment against bed checks.
30. F O N The patient resents inspection of lockers and bedside stands.
31. F O N The patient expresses resentment against rules and regulations.
32. F O N The patient demands personal articles of an unauthorized nature.
33. F O N The patient demands immediate service for his demands.
34. F O N The patient bothers the hospital staff with problems and demands.
35. F O N The patient calls unnecessarily for nurses and other personnel.
36. F O N The patient is demanding of attention.
37. F O N The patient doesn't seem to trust other people.
38. F O N The patient is unfriendly toward the hospital staff.
39. F O N The patient is disliked by other patients.
40. F O N The patient expresses prejudice against patients of other races.
41. F O N The patient shows contempt for the aides.
42. F O N The patient stirs up trouble on the ward.
43. F O N The patient is a sourpuss.
44. F O N The patient has temper tantrums.
45. F O N The patient is a general nuisance to others on the ward.
46. F O N The patient talks continuously and is hard to shake off.
47. F O N The patient monopolizes the attention of the special services personnel and/or volunteer workers.
48. F O N The patient goes over the head of immediate authority with his problems.
49. F O N The patient discusses treatment with other patients rather than with authorized personnel.
50. F O N The patient is an "I know it all" type.

Each patient in the reliability study was rated on every item. The nurse-raters indicated for each of their patients whether the specific behavior occurred frequently (*F*), occasionally (*O*), or not at all (*N*). A scoring system in which a scale value is assigned to each type of rating was used. An item was given a score of *0* for no occurrence, *1* for occasional occurrence, and *2* for frequent occurrence. A total "misbehavior" score was computed by totaling the scores for the 50 items. High total scores indicated poor ward behavior, whereas low total scores indicated good ward behavior from a staff point of view. In order to compensate for differences in individual rater's judgments of what constitutes "frequent," "occasional," and "not at all," the raw scores of the patients in each nurse's sample were transformed to standard scores.[2] This statistical procedure permitted a direct comparison of

[2] A patient's standard score gives one a direct knowledge of how his behavior compares with that of other patients. In statistical terms, the standard score of a patient indicates the deviation of his raw score from the mean raw score of his rater's sample in terms of the standard deviation of this sample. The formula for the type of standard score used in this study is:

$$\text{standard score } (T) = 50 + \frac{10\ (\text{raw score} - \text{mean})}{\text{standard deviation}}$$

In using the Ward Behavior Scale, raw scores may be used to evaluate a patient's behavior only if all patients are rated by a single judge. If more than one rater is involved, it is necessary to convert the raw scores in each judge's sample to standard scores (6, p. 131).

TABLE 1

COMPARISON OF INTER-RATER RELIABILITIES FOR TWO METHODS OF RATING WARD BEHAVIOR

METHODS	INTER-RATER CORRELATION
General questions	
Cooperation question	0.58
Bed-rest question	0.54
Ward Behavior Rating Scale	0.79

patients' scores and allowed the computation of an over-all correlation between the ratings of the head nurses and their ward nurses.

OBSERVATIONS

Inter-rater reliabilities for the total sample were computed for both rating methods, correlating the ratings of the head nurses with those of their ward nurses. The results are summarized in table 1.

A comparison of the two methods indicates that the Ward Behavior Scale yields more agreement between independent raters than does the method of employing general questions. The difference between the correlation obtained with the Ward Behavior Scale and that obtained with the Cooperation question is statistically significant ($P < 0.05$). This result might logically have been expected. The necessity of having to rate many specific overt actions rather than rating on the basis of over-all impressions should tend to minimize rater-introduced error. Pertinent to this is Cronbach's (6) observation that ratings based on incidental impressions are quite untrustworthy, whereas judgments based on systematic observations can be very reliable. Although the difference between the two methods was not quite of this order, nevertheless the use of questions calling for over-all impressions tends to yield data based more on incidental impression. Conversely, a number of ratings on specific actions more or less forces the rater to recall whatever actual behavioral observations have been made over the period of relationship with the patient.

It is believed that the agreement between the judges using the Ward Behavior Scale would have been higher had they received previous training with the scale. A greater familiarity with the items should result in an increased focusing of the rater's attention on the pertinent aspects of the patients' daily behavior. The acquisition of this common frame of reference for observing patients' behavior would probably result in increased agreement between observers using the scale.

In addition to the requirement of reasonably high agreement between raters using the Ward Behavior Scale, it was necessary to find out whether the scale yielded a stable measure of the patients' behavior. In other words, would the patients' scores come out the same on a second rating, or would they tend to fluctuate unpredictably? In order to test the stability of the scale, each of the nurse-raters was asked to rerate their patients twenty-four days later (it is assumed that the patients' behavior did not change significantly during this pe-

riod of time). A comparison of the first and second ratings yielded an average coefficient of stability of 0.89. The individual correlations ranged from 0.77 to 0.94. These figures support the belief that the Ward Behavior Scale yields reliably stable estimates of patients' behavior.

SUMMARY

In order for physicians, hospital administrators, and research workers in tuberculosis hospitals to deal effectively with the important problem of patients' ward behavior, it is necessary that they obtain an accurate picture of such behavior. The customary method of using the subjective impressions of staff members does not always yield reliable information. This paper describes the development of a Ward Behavior Rating Scale which provides a reliable and stable measure of ward behavior among tuberculous patients. The scale may easily be used by ward physicians, nurses, or other staff personnel who have occasion to observe the patients' behavior during a period of time.

SUMARIO

Escala para Apreciar el Comportamiento de los Tuberculosos Hospitalizados en las Salas

A fin de que los médicos, los administradores de hospitales y los investigadores de los hospitales para tuberculosos atiendan debidamente el importante problema del comportamiento de los enfermos en las salas, es necesario que obtengan un cuadro exacto de dicho comportamiento. El método acostumbrado de guiarse por las impresiones subjetivas de los miembros del claustro no aporta siempre datos fehacientes. Describe este trabajo la elaboración de una Escala de Justipreciación del Comportamiento en las Salas que ofrece una medición fidedigna y estable del comportamiento de los tuberculosos en las salas. La escala puede ser usada fácilmente por los médicos de sala, las enfermeras u otro personal técnico que tengan ocasión de observar la conducta de los enfermos durante cierto período de tiempo.

RESUME

Une échelle d'évaluation du comportement dans les salles du tuberculeux hospitalisé

Pour permettre aux médecins, aux administrateurs d'hôpital et aux investigateurs de traiter efficacement le problème important du comportement des malades dans les salles des services de tuberculose, il est nécessaire qu'ils obtiennent une image précise de ce comportement. La méthode habituelle, qui consiste à utiliser l'impression subjective du personnel médical de l'hôpital, ne donne pas toujours des renseignements sûrs. Cet article décrit la mise au point d'une échelle d'évaluation du comportement à l'hôpital, qui procure une mesure stable et valide du comportement dans les salles (ward behavior rating scale) du malade tuberculeux. Cette échelle peut être utilisée avec facilité par les médecins, les infirmières ou tout autre personne du service ayant l'occasion d'observer le comportement des malades pendant un certain laps de temps.

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A PLANNED PROGRAM OF EDUCATION VERSUS INCIDENTAL INSTRUCTIONS FOR PATIENTS RECEIVING TREATMENT FOR TUBERCULOSIS[1]

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INTRODUCTION

The purpose of the present study was to determine whether or not there are differences between patients who are receiving treatment for tuberculosis in different hospitals. The difference investigated was related to the knowledge and understanding that the patients have about their disease, its treatment, and their part in the treatment. If there is a difference in the amount of knowledge and understanding, a question to be considered is: what might that difference mean to the patients? One might ask further: in the light of the acknowledged need for the patient to know about his disease in order that he may more fully cooperate in his cure and in helping himself get well, do all patients hospitalized for tuberculosis assimilate a reasonably comparable body of knowledge and understanding about their disease? A hospital which did not use modern surgery or recently discovered drugs for the treatment of its patients might be criticized. The failure to furnish the patient with adequate instructions might warrant similar criticism.

PLAN OF INVESTIGATION

For the purposes of the investigation, two tuberculosis hospitals, the Detroit (Michigan) Tuberculosis Sanatorium and Glen Lake (Minnesota) Sanatorium, which possess many matching elements were selected. These hospitals were comparable in their nursing and medical staffing patterns, with similar ratios of each professional group to the numbers of patients. Each hospital draws its patients from a large industrial area and each uses the most modern tools and techniques of therapy for the treatment of the patients. Detroit Tuberculosis Sanatorium is a private hospital and Glen Lake Sanatorium is a county institution, but the patient population of each hospital was comparable in the percentages of private and county cases.

Besides selecting matched hospitals, the records of all patients were reviewed and matched samples of patients were selected. The seven attributes possessed by the patients or related to their individual therapy which might have influenced the replies of the patients and which it was possible to match in the two groups were:

1. Type of therapy in addition to bed rest
2. Number of hospitalizations for tuberculosis
3. Date of original diagnosis
4. Marital status
5. Sex
6. Age
7. Diagnosis at the time of the present hospitalization

[1] This study was done for a doctoral dissertation at the University of Michigan.

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Familiarity with the English language was not used as an element for matching, but in each group there was one war bride. One had been here two years and the other one and one-half years and each spoke and seemed to understand English very well. All of the other patients had been born in the United States.

One conspicuous difference between the two institutions which influenced the writer in the selection of these two hospitals for study was the existence at Glen Lake of a coordinated patient education program with the necessary accompanying in-service education program for the professional staff and the lack of organized programs of this type at Detroit.[3]

The more homogeneous the two groups of patients considered for such a study, the more alike the replies to the measuring instrument are apt to be. By having the patients *matched* for factors which could have influenced their replies, it can be said that these factors were controlled and could not have caused a difference in the replies. Conversely, a factor which *does not match* in the two groups conceivably could make a difference. One study cannot result in the proof that the single factor of difference influenced the differences between the two groups of patients, but individual replies, some of which will be delineated in the observations, do indicate differences and possible reasons for the differences.

Two matched groups of 40 patients from each hospital were selected and individually interviewed. A schedule of open-end questions was developed and used as a guide for the interviews and as a record for the data obtained. The patients seemed to take a genuine interest in the interviews and all gave attentive thought to each question and answer. The writer believes that the patients' replies represent fully each individual's knowledge and opinion of the particular subjects discussed.

Because, thus far, no statistical test has been devised which would allow a statement of proof related to the data collected in this investigation, the only statistical test applied was the chi-square test for statistical significance, which can be used as a standard of reference to indicate when differences in replies might have been due to chance ("chance," many small factors) or when the differences were not likely due to chance. It was determined from a table of levels of significance that a chi-square of 3.84 would indicate admission of chance being a possible determining factor to the 5 per cent level in a random sample. Knowing that the attributes which were controlled could not have influenced the results, it is freely admitted that chance could have been the influencing factor in the replies of 5 per cent of the patients when the chi-square test showed 3.84 or more. When the results of the use of the chi-square were less than 3.84, they may have been due to pure chance.

OBSERVATIONS

For the purpose of analyzing the data, the questions were grouped under five major headings:

What the patients know about tuberculosis

[3] At the outset and before consideration of the two hospitals and the data obtained from each, it should be clearly stated that every possible effort was expended to treat all aspects of the study with objectivity. Neither the description of the present practice, the statement of the findings, nor the citation of the significance of the findings in their relation to tuberculosis therapy are meant to imply a judgment of the current program in either institution. The writer believes that there is considerable value in not having to try to maintain anonymity in the study and appreciates the permission granted by the administrators of the two hospitals to indicate freely the sources of the data.

What the patients know about their own conditions
What the patients understand about their own part in their treatment
What the patients do in relation to accepting treatment
The patients' attitudes toward their illness and treatment

In the first analytical approach to the data, counts were taken of the number of patients from each group who gave a correct or satisfactory reply to each question which could be classified in the five groupings. Whenever a difference between the number of correct replies appeared, a chi-square test was applied to determine whether or not the difference was significant or could have been due to chance.[4]

In each grouping there were questions which demonstrated differences between the two arrays of respondents. The differences revealed were significant by statistical analysis and had practical implications. In order to illustrate these variations in replies, a representative question from each category is listed and both of these aspects of the replies are discussed.

A representative question from the area of the patient's knowledge of the disease in general is, *"Could you tell me what causes tuberculosis?"* The tabulated results for this question showed that 8 patients at Detroit, in contrast to 27 patients at Glen Lake, stated that a germ was the cause of the disease. Since lowered resistance as a contributing cause of tuberculosis is given such prominence in much of the literature written for patients, those who gave this as a reply to the question were credited with knowledge of the subject. This changed the tabulations to answers of 25 patients at Detroit and 37 patients at Glen Lake who knew the cause of their illness. The chi-square test applied to these second counts yielded 7.86, which indicated that the difference was significant. From a practical standpoint, the apparent ignorance of the fact that a germ is the cause without which there would be no disease precipitates concern for the understanding that these patients might have of precautionary measures necessary to protect other people. This area of concern was investigated further in other questions, and the same difference between the two groups continued to be demonstrated. The data obtained through these and other questions about the disease indicate a difference between the two groups of patients concerning their knowledge of the disease itself.

A representative question from the category of what the patient knows about his own condition revealed a difference between the two groups of patients and also indicated a difference in the amount of information about their condition which had been imparted by the doctors in the two institutions to their patients. In reply to the query, *"Could you tell me how long you were in the hospital before you knew approximately the length of time you will have to be here?"*, 17 patients at Detroit in contrast to 33 at Glen Lake (chi-square 12.53) stated that they knew before they were in the hospital two months that their treatment would

[4] The results of these tabulations and descriptions of the programs for patient education for the patients in each hospital, along with one which is suggested as an adequate program, appear in the complete report of the study, which is available on loan from the microfilm library of the University of Michigan, Ann Arbor, Michigan.

be a long-time procedure, and hence, planned that they would be in the hospital at least a year. Individual replies to this question further illustrated a contrast between the two hospitals in the practice of the doctors in telling patients about their conditions and what to expect in regard to their treatment. All but 2 of the patients at Glen Lake, who said that they knew before they were in the hospital two months that they would have to remain in the hospital for a year or more, stated that the doctor had told them. Many of these patients stated that they had been in the hospital less than two weeks when they were told. This information was forthcoming when the doctor showed them their first roentgenograms and discussed their entire treatment plan with them. In contrast to this, 7 of the 17 patients at Detroit who did know before two months of hospitalization that their treatment would be lengthy stated that they had learned this from other patients and that their doctor had "never" told them.

This question also elicited replies which indicated feelings of concern and some of positive resentment at not having been told what to expect. One patient's statement to this effect and one which is almost the exact quotation of 5 Detroit patients is:

> I thought I would be here three months. When that time came I knew it would be longer. I have been here eight months now, and I still do not know how long I'll have to be here. They just go on keeping me here, but they never tell me anything.

Typical of the twenty-eight questions classified in the third area of inquiry, the patient's understanding of his part in the treatment, is the query, "*Do you think that a patient can have too much bed rest?*" The responses to this question were also typical in that they represented the differences shown by the two sets of respondents to many of the questions in the category. Eleven of the patients at Detroit and 27 at Glen Lake (chi-square 11.27) stated that one could not have too much bed rest. Many individual replies portrayed the practical importance in the understanding, or lack of it, related to this phase of tuberculosis therapy. Ten patients at Detroit stated that they took a walk in the corridor once or twice a day so that they would not become too weak from bed rest. Several of them stated that they did this on the recommendation of other patients. Further consideration of the significance of replies to this question relates to one which is representative of the fourth category and will be detailed with it.

This category of questions investigating the patients' activities relating to their treatment includes the query, "*Will you tell me how much time you actually do spend out of bed each day?*" In reply, 13 patients at Detroit and 13 at Glen Lake (chi-square 20.63) stated that they spent only the amount of time out of bed that the doctor had ordered. The practical importance of these replies looms large when one considers the concept of bed rest as therapy in relation to the utilization of this therapeutic measure by the patients at Detroit and at Glen Lake. The replies confirm the point of view of the doctor at Glen Lake who described the bed rest policy of that institution. He stated that the staff at Glen Lake believes that in many instances the patients could safely be allowed one bathroom privilege a day, but that they had learned from experience that,

if they permit this as a matter of policy, the patients soon take greater privileges. At Detroit, 2 patients who were on strict bed rest and 2 who were permitted only one bathroom privilege a day were among the 13 who followed their doctor's recommendations. The 27 patients who were up more than the doctor ordered included all patients allowed full bathroom privileges.

In other words, as has long been recognized, the practice of treatment which inflicts less restriction on the patients' activities does not necessarily result in keeping them on a rest regimen. More patients with liberal exercise programs took "privileges" beyond the doctors' orders than patients who were restricted to no out-of-bed activities. There were other questions which also revealed that patients at Glen Lake are given more specific instructions concerning their bed rest. These two questions reveal that there is a marked difference between the two groups of patients in both their understanding of their own part in the treatment and their activities in relation to the treatment.

The differences between the two groups of patients continued to be illustrated by replies to questions pertaining to the patients' attitudes in regard to their illness and treatment. Perhaps the most representative question of this classification is, "*Do you think that you would accept any operations or other treatments which your doctor suggested would help you?*" The differences in attitudes displayed by the two groups of patients seem definitely attributable to the amount of information which the patients had been given about their treatments and to the kind of understanding which they possessed in relation to them. There were only 19 patients at Detroit, in contrast to the 40 patients interviewed at Glen Lake (chi-square 10.96), who stated, without qualification, that they would accept any treatment which the doctors might suggest. There were only 6 at Detroit who declared that they definitely would not accept any surgical therapy, but there were 15 who placed elaborate qualifications on the conditions under which they would accept. Some of the typical statements were:

> I would have to know a lot more about it, and I know that I could not learn that from these doctors. No, I don't think I'd have any surgery.
>
> They would have to show me my X-rays and explain to me what it was all about. They certainly haven't told me enough so far to get me to take any operation.
>
> I'd take any other treatment, I guess, but none of those operations.
>
> I wouldn't have an operation. I have seen too many bad results. Once they start, it means one operation after another. Something goes wrong in the first and then they have to do a second and a third to try to fix it. No, I just wouldn't let them get started.

Replies to this question seem to furnish concrete evidence that there is a definite difference between the comprehension and consequent attitudes of those at Detroit and Glen Lake about surgical therapy for themselves.

The foregoing discussion illustrates some of the typical queries used and the responses obtained in the collection of the data for the study. A brief summary of the results from all of the questions classified in the five areas of study shows that sixty-five questions yielded statistically significant, and not chance, differences between the two groups of patients. There were fifty-six questions in the five categories which did not yield statistically significant differences. Never-

theless, there were only three questions to which more patients at Detroit than at Glen Lake were able to give a reply judged appropriate for the question. With these three exceptions, of the one hundred and twenty-one questions analyzed, whenever there was a difference between the two groups, whether the difference was statistically significant or whether it might have been due to chance, the patients at Glen Lake more frequently answered appropriately.

The foregoing analysis considered the *number of patients* giving specified answers to the designated questions. A second approach to the data was made whereby counts were taken to determine the *number of correct or favorable replies* given by each patient. For this analysis, one hundred and eleven questions were classed as appropriate and distributed under appropriate headings, which are indicated by the titles of the four tables.

The tabulations in each table speak for themselves, but a chi-square test, the only valid statistical test available for these data, was again used as a standard of reference. The test was applied to the totals of the numbers of patients giving acceptable replies to more than half of each group of questions. In each instance

TABLE 1

NUMBER OF PATIENTS FROM EACH INSTITUTION WHO GAVE SPECIFIED NUMBERS OF CORRECT ANSWERS TO SIXTY QUESTIONS CONCERNING KNOWLEDGE OF THE DISEASE

INSTITUTIONS	NUMBER OF QUESTIONS CORRECT					TOTAL PATIENTS
	10–19	20–29	30–39	40–49	50–60	
Detroit	3	9	7	17	4	40
Glen Lake	0	0	4	11	25	40

TABLE 2

NUMBER OF PATIENTS FROM EACH INSTITUTION WHO GAVE SPECIFIED NUMBERS OF CORRECT ANSWERS TO TWENTY-NINE QUESTIONS WHICH INDICATE THAT PATIENTS WERE GIVEN SPECIFIC INFORMATION

INSTITUTIONS	NUMBER OF QUESTIONS CORRECT					TOTAL PATIENTS
	8–12	13–17	18–21	22–25	26–29	
Detroit	9	15	10	5	1	40
Glen Lake	1	2	4	14	19	40

TABLE 3

NUMBER OF PATIENTS FROM EACH INSTITUTION WHO GAVE SPECIFIED NUMBERS OF CORRECT ANSWERS TO NINE QUESTIONS CONCERNING ACTIVITIES FAVORABLE TO PATIENT'S CURE AND TO THE PROTECTION OF OTHERS

INSTITUTIONS	NUMBER OF QUESTIONS CORRECT				TOTAL PATIENTS
	1–3	4–5	6–7	8–9	
Detroit	10	15	12	3	40
Glen Lake	0	2	10	28	40

TABLE 4

NUMBER OF PATIENTS FROM EACH INSTITUTION WHO GAVE SPECIFIED NUMBERS OF CORRECT ANSWERS TO THIRTEEN QUESTIONS WHICH INDICATE A FAVORABLE ATTITUDE TOWARD THEIR ILLNESS AND TREATMENTS

INSTITUTIONS	NUMBER OF QUESTIONS CORRECT					TOTAL PATIENTS
	1-4	5-7	8-9	10-11	12-13	
Detroit	3	5	10	14	8	40
Glen Lake	1	0	2	6	31	40

these tabulations significantly reiterated the results portrayed by the first analysis of the data.

DISCUSSION

All evidence indicates an affirmative answer to the question: Are there differences with respect to knowledge of tuberculosis between the patients receiving treatment at one hospital and those receiving treatment at another? Whether such differences can be related to the extent to which organized educational programs are in operation at individual institutions has not yet been established. As has been pointed out above, a single study cannot furnish the evidence for the answer to this most important question. Nevertheless, the data from the present study indicate a marked difference between these two particular groups of patients in respect to what they had been told about their illness and what they knew about it. Obviously, the element of difference between these two hospitals, in terms of programs for teaching patients about their illness, might not necessarily be the reason for the difference found in the patients' knowledge and consequent attitudes. Patients who are fortunate enough to receive adequate instructions on an incidental basis well might have equal or greater knowledge about their illness than patients who receive instructions in a planned program. Nevertheless, in the limited experience of the present writer, consisting of intimate knowledge of the programs in ten tuberculosis hospitals, it has been observed that a planned, coordinated program for patient education seems to create an atmosphere which permeates the whole hospital. As a result, all of the personnel take a more active part in teaching the patients than in a hospital in which patient teaching is limited to incidental episodes of instruction. Admittedly, this is not necessarily a universal phenomenon, but the writer has repeatedly found it to be the case, and the present study offers convincing evidence that in this one instance it definitely was so. Because of this experience and because the differences between these two groups were so marked, it is believed that, if other studies in other hospitals were conducted in similar vein, the same differences would be revealed.

On the basis of this experience, certain definite impressions have been found with respect to the questions proposed at the beginning of this report. It appears that patients who receive more adequate instructions about their illness and have a better understanding of it seem to have attitudes which enable them to

cooperate more fully in their treatment. All patients hospitalized for tuberculosis do not possess comparable understanding about their disease and what they can do to help themselves get well. A further question might be asked: "*What can be done to give all patients more help in this important area?*" The evidence related in the present report seems to supply the answer: a planned, coordinated patient education program.[4]

SUMMARY

A study was conducted to determine whether or not there was a difference in patients hospitalized in two different tuberculosis hospitals. Significant differences were found with respect to the knowledge which patients have about the disease and about their own particular conditions, their understanding of their part in their treatment, their physical activities related to their cure, and their attitudes toward their treatment. The two hospitals selected for investigation were similar in many elements but differed in their plans, programs, and practices for giving patients information about their illness.

SUMARIO

Un Plan Meditado de Enseñanza en Terapéutica Tuberculosa Contrastado con Instrucciones Fortuitas a los Enfermos

Tratóse en este estudio de determinar si había o no alguna diferencia entre los enfermos hospitalizados en dos distintos hospitales para tuberculosos. La diferencia investigada se relacionaba con los conocimientos poseídos por los enfermos acerca de su enfermedad y acerca de su situación propia, con su comprensión del papel que les corresponde en su tratamiento, con sus esfuerzos físicos en relación con su curación y con su actitud hacia el tratamiento. Los dos hospitales escogidos para investigación eran semejantes en muchos elementos, pero discrepaban en sus planes, obras y prácticas en lo tocante a suministrar a los pacientes información con respecto a su dolencia.

RESUME

Directives selon un programme défini versus instructions fortuites (incidental) en phtisiothérapie

Une étude a été entreprise pour déterminer l'absence ou la présence de différences entre les malades hospitalisés dans deux différents services de tuberculose. L'investigation de ces différences concernait: les connaissances que les malades possédaient sur l'affection et leur état particulier: leur compréhension de la valeur de leur participation à leur traitement; leurs activités physiques en relation avec leur cure et leur attitude vis-à-vis de leur propre traitement. Les deux hôpitaux choisis pour l'investigation étaient semblables à de nombreux points de vue, mais ils différaient quant au plan de traitement, aux programmes et aux méthodes utilisés.

PRELIMINARY STUDIES ON ARTIFICIAL ACTIVE IMMUNIZATION OF GUINEA PIGS AGAINST RESPIRATORY CHALLENGE WITH COCCIDIOIDES IMMITIS[1]

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INTRODUCTION

Coccidioidomycosis, a fungus infection caused by *Coccidioides immitis*, is endemic in certain areas of the arid southwestern region of the United States (1). A high percentage of the resident population and many transients through these areas have contracted the disease which, in a majority of the cases, is subclinical or a mild to severe, self-limited benign pulmonary infection (2). As a result of primary infection, a sensitivity to the fungus or its products develops which can be demonstrated by a positive skin reaction to coccidioidin (3).

Competent investigators (4, 5) have observed that persons who have had a primary infection, as determined by a positive skin reaction, possess an immunity to exogenous reinfection. Such observations suggested the feasibility of artificial active immunization against coccidioidomycosis to protect susceptible newcomers in endemic areas.

As a spherule antigen could be obtained from the yolk sac of embryonated hens' eggs (6), this material was used in an attempt to produce artificial active immunity in guinea pigs against an experimentally induced pulmonary infection with an aerosol of *C. immitis* arthrospores.

MATERIALS AND METHODS

The spherule stage of *C. immitis* was cultured in the yolk sac of the embryonated hen's egg as described in a previous report (6). A saline suspension of spherules was heated at 60°C. for thirty minutes, tested for sterility, and preserved with 1:10,000 Merthiolate.® A standard concentration of 1:50 by volume of this spherule material was used for immunization.

White guinea pigs weighing 300 to 500 gm., obtained from Carworth Farms, New City, New York, were used in the experiments. The immunization schedule consisted of five intramuscular injections of 0.5 ml. of the spherule suspension at weekly intervals. The animals were challenged with an aerosol of *C. immitis* arthrospores eight to twelve weeks after the last immunizing injection.

The aerosol was prepared from dried cultures of *C. immitis* according to the method of Cronkite and Lack (7). The harvested spores and mycelial fragments were ground in a mortar and dried for several days in a desiccator. Plate counts of this material were made by suspending 10 mg. in 10 ml. of saline and plating 0.1 ml. amounts of appropriate dilutions on Sabouraud's dextrose agar plates.

A large tank of 5,000 liters capacity was used as the exposure chamber. Humidity and

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temperature were adjusted to simulate environmental conditions of an arid, warm climate. A weight of aliquot aerosol material was placed in a discharge apparatus designed to explode the spores into the tank with great force, and an overhead fan within the tank aided in the dispersal of the aerosol. On two occasions, *Experiments 1* and *2*, aerosol inocula contained 19×10^6 viable particles, respectively. No attempt was made to determine the number of spores inhaled by the animals during an exposure period of one hour.

The coccidioidin skin test was used to follow the development of sensitivity in animals undergoing immunization and in the control animals after exposure to the aerosol. The coccidioidin, Lot 29-31, was kindly supplied by Dr. C. E. Smith.

In a preliminary trial, *Experiment 1*, only a gross examination of the lungs was made, and bits of the tissue were cultured to establish infection by *C. immitis*. On the basis of the findings in this experiment it was assumed that the spread from this focus would manifest itself by disease in regional lymph nodes and subsequent dissemination. The lungs of all subsequently exposed guinea pigs, therefore, were injected with 10 per cent formalin, and the entire thoracic contents were removed for gross and microscopic examination. It was believed that any protection which the animal acquired through the treatment would manifest itself in a decreased number of foci of infection in the lung. This assumption was the basis for the categories given below:

Mild infection = 1 to 5 macroscopic (1 to 2 mm.) tubercles in both lungs; no pneumonia

Moderate infection = 6 to 10 tubercles in both lungs or less than 5 small foci (less than 3 mm.) of pneumonia

Severe infection = more than 11 tubercles or pneumonia greater than above

Positive lymph nodes = focal or diffuse granulomatous lesions with or without organisms

Number of organisms per focal lesion per slide = many, more than 20; moderate, 11–20; few, 1–10

The above definitions will be used in their respective meanings in the remainder of this paper.

RESULTS

Experiment 1: Sixteen guinea pigs were injected with spherule material as described above. They developed a positive skin reaction to an intradermal injection of 0.1 ml. of a 1:50 dilution of coccidioidin four weeks after the initial injection. At this time, 13 of 16 treated guinea pigs had positive skin reactions, while 16 nontreated control animals had negative reactions. Both groups were exposed simultaneously to the aerosol for one hour. Two weeks after exposure the skin reactions of the treated animals were markedly depressed, while 9 of 16 control animals had developed positive reactions. Six weeks after exposure, at the termination of the experiment, the treated animals still retained a low degree of sensitivity, at which time the majority of the controls had developed a strongly positive reaction. Both groups of animals appeared in good health just prior to autopsy.

When the control animals were sacrificed and examined macroscopically, several were found to have raised, whitish tubercles on the lung surface and a few were seen to have areas of lung involved with greenish gray, poorly demarcated foci resembling abscesses. The degree of macroscopic disease was judged to be less in the treated groups of animals. Cultures of the lung tissue from all of the treated and all of the control animals were positive for *C. immitis*.

Experiment 2: Twenty-four guinea pigs were treated with spherule antigen.

These animals developed a positive skin reaction to coccidioidin during the fourth week, at which time 22 of 24 animals had positive reactions. Twenty-four control guinea pigs had negative reactions to the same coccidioidin skin-test dose.

All 48 guinea pigs were exposed simultaneously to the aerosol for one hour. Six animals from each group (treated and controls) were sacrificed at the end of one and two weeks after challenge in order to follow the early development of their infection. The remaining 12 animals from each group were sacrificed at the end of six weeks.

The skin reactions were depressed in the treated animals one week after exposure. Several controls showed a slight positive reaction at this time. Unfortunately, the supply of coccidioidin provided by Dr. C. E. Smith was exhausted and an inferior sample had to be used for future tests. Subsequently, the skin reactions of the controls remained depressed and never developed the degree of sensitivity of the control animals in *Experiment 1*. The pathologic findings of this group of guinea pigs are presented in table 1.

In this table, *Group I* represents the guinea pigs sacrificed one week after exposure; *Group II* represents the guinea pigs sacrificed two weeks after exposure; and *Group III* represents those guinea pigs sacrificed six weeks after exposure. It will be seen that no organisms could be demonstrated microscopically in either treated or control animals of *Group I*. As cultures were obtained from lung tissue of all animals examined in *Experiment 1*, it is assumed that positive

TABLE 1

EXPERIMENT II: PATHOLOGIC FINDINGS IN TREATED AND CONTROL GUINEA PIGS CHALLENGED WITH AEROSOL OF COCCIDIOIDES IMMITIS

	GROUP I*		GROUP II		GROUP III	
	Treated	Controls	Treated	Controls	Treated	Controls
No infection	5	0	2	1	5	1
Mild infection	1	4	1	1	4	0
Moderate infection	0	2	2	2	2	3
Severe infection	0	0	1	2	1	8
Many organisms, lung	0	0	0	0	1	3
Moderate organisms, lung	0	0	0	1	1	3
Few organisms, lung	0	0	1	2	3	4
Lymph nodes, positive	0	0	0	0	3	11
Many organisms, lymph nodes	0	0	0	0	0	2
Moderate organisms, lymph nodes	0	0	0	0	0	4
Few organisms, lymph nodes	0	0	0	0	3	5
Extrathoracic infection	0	0	0	0	0	2

* *Group I:* 6 controls, 6 treated animals; sacrificed one week after exposure.
Group II: 6 controls, 6 treated animals; sacrificed two weeks after exposure.
Group III: 12 controls, 12 treated animals; sacrificed six weeks after exposure.

cultures also could have been demonstrated in this group of animals. Five of the treated animals were free of infection and one had mild infection. In the control animals of this group, however, 4 had mild infection and 2 had moderate infection. All 6 animals of this group had tubercles.

In *Group II*, animals sacrificed two weeks after exposure, the difference between the treated and the control animals was not striking but was nevertheless evident. Two of the treated animals had no infection; the 4 remaining animals showed mild, moderate, and severe infection. One of the control animals of this group was free of infection. The infection present in the remainder seemed to be more intense than that in the treated group. Only slight significance is attached to the fact that organisms were seen in 3 of the controls, compared with one animal in the treated group.

A more striking and significant difference was observed, however, in the animals in *Group III*, which were sacrificed six weeks after exposure. Only three treated animals had moderate to severe infection, while 11 of the controls could be placed in these categories. The same numerical difference also should be noted concerning the spread of infection to the lymph nodes. Dissemination beyond the thoracic cavity could not be demonstrated in the treated animals but was demonstrated in 2 of the control group. The results in these three groups of 12 animals each, treated and control, would indicate that injection of killed spherule material into guinea pigs prior to exposure to an aerosol of *C. immitis* minimized pulmonary and regional lymph node infection by this fungus.

DISCUSSION

Progressive disease, coccidioidal granuloma, is analogous with primary tuberculosis of infancy. The disease occurs in some persons, particularly in nonwhites, who fail to develop immunity to a primary infection which continues to disseminate during a period of weeks, months, and occasionally years (2, 8). The desirability of an artificial active immunization against a primary infection which may become progressive becomes of paramount importance for the protection of susceptible persons before entry into known endemic areas. That such an immunization should be possible is suggested by epidemiologic data which indicate that a naturally acquired primary infection confers immunity to exogenous reinfection (4, 5, 8).

Experimental studies with laboratory animals, however, have been concerned for the most part with the development of the spherule stage of *C. immitis* (9) and the response of infected (10) or immunized animals (3, 11) to an intradermal injection of coccidioidin. In one instance, previously infected animals were challenged with living organisms for the purpose of investigating their reaction to the material. In these experiments, Negroni and Bonfiglioli (12) reported that organisms injected into the testes of previously infected guinea pigs were quickly destroyed and eliminated from the tissues. They compared this reaction to Koch's phenomenon in the tuberculous guinea pig.

The pathologic findings in the present report also suggest acquired resistance to a pulmonary infection by *C. immitis* in sensitized guinea pigs. Pulmonary

infection in the sensitized animals not only appeared less frequently but also was less severe than in the control animals. The most dramatic differences in host response to infection were noted in the animals sacrificed six weeks after exposure to an aerosol. The severity of the infection as well as the incidence of positive lymph nodes and the number of organisms in both the nodes and lungs were much higher in the control animals. It would seem, therefore, than an injection of sufficient antigen to produce a positive skin reaction also produced a relative immunity sufficient to inhibit lymphatic dissemination or enabled the animal to destroy the organisms within the lymph nodes before a tissue reaction could take place.

SUMMARY

Guinea pigs were sensitized to coccidioidin by repeated intramuscular injections of a heat-killed, saline suspension of spherules. Increased resistance to an aerosol of living arthrospores of *C. immitis* was suggested by macroscopic and microscopic comparison of infection in sensitized and nonsensitized guinea pigs.

SUMARIO

Estudios Preliminares de la Inmunización Artificial Activa de los Cobayos Contra la Provocación Respiratoria con Coccidioides Immitis

Se sensibilizó a cobayos a la coccidioidina por medio de repetidas inyecciones intramusculares de suspensión salina de esférulas matadas al calor. La comparación macro y microscópica de la infección en cobayos sensibilizados y no sensibilizados sugería un aumento de la resistencia a un aerosol de artrosporas vivas del *Coccidioides immitis*.

RESUME

Etude préliminaire sur l'immunisation artificielle active des cobayes contre l'attaque des voies respiratoires par les Coccidioides immitis

Des cobayes ont été sensibilisés à la coccidioidine par l'injection intramusculaire répétée d'une suspension saline de sphérules tués par la chaleur. La résistance accrue à un aérosol d'arthrospores vivantes de *Coccidioides immitis* a été suggérée par l'étude comparative macroscopique et histo-pathologique de l'infection chez le cobaye sensibilisé ou non-sensibilisé.

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Case Reports

PERIPHERAL NEURITIS IN TUBERCULOUS PATIENTS TREATED WITH ISONIAZID[1]

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It is well known (1, 2, 3) that the principal toxic effect, thus far observed, of isoniazid on human beings is the production of peripheral neuritis. The purpose of this paper is to report experiences of the present writers in this connection.

Among approximately 500 tuberculous patients, each treated for at least nine months[2] with isoniazid by mouth in doses of 4 to 8 mg. per kg. per day, without vitamin supplements of the B-complex group, 8 cases of peripheral neuritis attributable to isoniazid have been encountered.

Pertinent observations on these patients and on the possible use of pyridoxine in the control of this syndrome are described in the accompanying figure 1.

CASE REPORTS

Case 1. The patient, R. K., had moderately advanced pulmonary tuberculosis, with a history of abdominal discomfort for nine months before an exploratory laparotomy with inconclusive results in July, 1952. She was admitted to this hospital soon thereafter in a state of severe emaciation and subileus. Laparotomy (May, 1953) showed extensive pneumatosis cystoides intestinalis (*hominis*) of the entire jejunum and ileum. Resection of 60 cm. of the proximal ileum, the site of three marked strictures, was performed. Gastrointestinal function and nutrition improved from this point on. Cortisone in initial dosage of 300 mg. per day, decreasing to a maintenance dose of 25 mg. per day, was given from August 31 through November 15, 1953. During this period the patient was "covered" by isoniazid in a dosage of 12 mg. per kg. per day. It was toward the end of this period that the patient developed mild symptoms of peripheral neuritis: tingling in hands and feet with some numbness in both feet. There were no motor or trophic disturbances at any time. Both isoniazid and cortisone were discontinued, and the patient lost her neuritic symptoms entirely within eight weeks.

Case 2. The patient, G. B., had far advanced pulmonary tuberculosis with previous thoracoplasty. He had a muscular atrophy affecting mainly the quadriceps of the left thigh, which represented a residual of anterior poliomyelitis in childhood. The patient's neuritic symptoms after treatment with isoniazid were limited to the left foot and the lower left calf; these consisted only of minor sensory disturbances. The symptoms cleared markedly on discontinuation of isoniazid therapy and administration of pyridoxine hydrochloride. Three weeks later, when isoniazid was administered at the same dosage, with concomitant pyridoxine, no neuritic symptoms recurred.

Case 3. The patient, D. M., had suffered from moderately advanced pulmonary tuberculosis for many years. When he belatedly reported symptoms of peripheral neuritis, he had quite

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[2] Except for the patients who developed neuritis during shorter periods of administration of isoniazid (*see* figure 1).

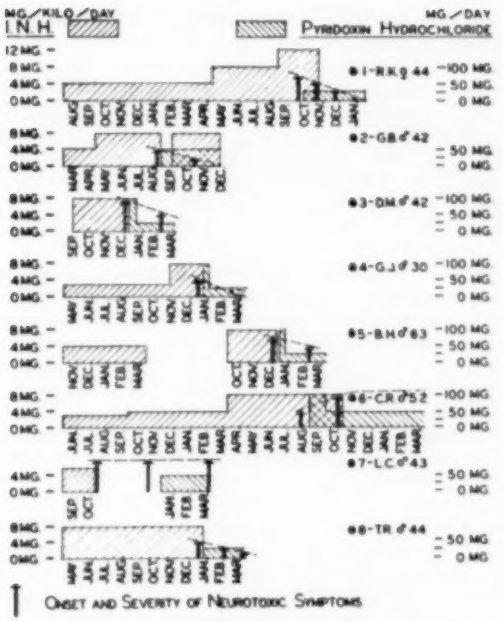

FIG. 1. Pertinent clinical data on the appearance of neurotoxic symptoms in tuberculous patients treated with isoniazid for different periods and at different dosage levels during 1953–1954. The first arrow indicates in each case when the symptoms of peripheral neuropathy appeared; the height of an arrow indicates their relative severity.

severe sensory impairment in both feet, legs, and hands; he also complained of some "weakness" in both legs. No objective pathologic findings were discovered in the motor function of the lower extremities. This patient's symptoms abated on discontinuation of isoniazid and the administration of pyridoxine.

Case 4. This patient, G. J., with minimal pulmonary tuberculosis was a very moderate alcoholic but a neurotic and highly suggestible person, and had previously been on streptomycin therapy, which was discontinued because of severe paresthesias over the area served by the two lower branches of the trigeminal nerve. His neurotoxic symptoms, following eight months of isoniazid therapy (six months at 3 mg. per kg. per day, and the last two months at 8 mg. per kg. per day) were mild in character. They improved quickly on discontinuation of isoniazid and institution of pyridoxine treatment. This patient had suffered many years previously with tuberculous enteritis and peritonitis. However, he had made a good recovery and had regained his usual weight before isoniazid therapy was begun.

Case 5. The patient, B. H., represented a case of long-standing alcoholism with moderately advanced, cavitary pulmonary tuberculosis. Mild neurotoxic symptoms of the peripheral sensory system developed after three months of 8 mg. of isoniazid per kg. per day (this patient had had, a year earlier, six months of 4 mg. of isoniazid per kg. per day). The symptoms improved somewhat after discontinuation of isoniazid and institution of pyridoxine treatment.

Case 6. The patient, C. R., had far advanced pulmonary tuberculosis with cor pulmonale and chronic alcoholism. He developed mild symptoms of neurotoxicity after twelve months of isoniazid therapy (8 mg. per kg. per day for the last four months). Nevertheless, because of the extensive character of his pulmonary lesions and sputum persistently positive for tubercle bacilli, isoniazid was continued at the same dosage for another three months, with the simultaneous administration of pyridoxine hydrochloride, 100 mg. per day by mouth. During this three months' period of "combined" therapy, however, his symptoms became so severe that, in spite of the absence of objective neurologic findings, isoniazid therapy was discontinued. His symptoms have improved but little during five subsequent months of pyridoxine treatment.

Case 7. The patient, L. C., had moderately advanced pulmonary tuberculosis and was a moderate alcoholic. He had been treated for only two months with isoniazid at 6 mg. per kg. per day when he developed acute, severe neurotoxic symptoms: stocking-type hypesthesia of both upper and lower extremities, severe ataxia, and some weakness of the left arm and leg, with nystagmus. Isoniazid therapy was discontinued. Neurologic investigations failed to reveal any definite organic disease of inflammatory or degenerative type. Serologic tests for syphilis were negative. The patient was admitted to this hospital after the onset of his symptoms. Pyridoxine therapy (50 mg. intramuscularly daily) for three months failed to alleviate his neurotoxic signs and symptoms; on the contrary, the ataxia and weakness of the left extremities has increased.

Case 8. The patient, T. R., had far advanced pulmonary tuberculosis with previous thoracoplasty and was a chronic alcoholic. This patient manifested moderately severe neurotoxic (sensory only) disturbances after eight months of isoniazid therapy at 8 mg. per kg. per day. Afterdiscontinuation of isoniazid and institution of pyridoxine treatment, he improved, and, after three months, was practically free of symptoms.

COMMENT

It is noteworthy that 7 of these 8 patients had histories of alcoholism. The one patient (R. K.) who had no history of alcoholism had a disease, "pneumatosis cystoides intestinalis," which resulted in poor gastrointestinal function and chronic malnutrition. The clinical appearance of peripheral neuritis has, in the writers' experience, been observed primarily in alcoholics. Nevertheless, 4 patients with clear-cut alcoholic histories failed to develop any evidences of peripheral neuritis on long-continued isoniazid therapy even though their alcoholic intake was only slightly diminished and they received no supplementary vitamins. Thus, overindulgence in alcoholic beverages would not, alone, appear to be sufficient to predispose a person to peripheral neuritis on isoniazid therapy.

Six of the 8 patients who developed peripheral neuritis showed striking abatement of their symptoms on discontinuation of the isoniazid and the administration of pyridoxine[3]. Furthermore, in 2 cases, peripheral neuritis, associated with chronic alcoholism and present before initiation of isoniazid therapy, was not aggravated by isoniazid therapy (8 mg. per kg. per day) with concomitant administration of pyridoxine (25 mg. per day) during periods of more than six months. The rational basis for the administration of pyridoxine was as follows: Pyridoxine has been shown to antagonize the antimicrobial activity of isoniazid against many bacterial species, except for tubercle bacilli (4). It has been reported (5) that pyridoxal, an aldehydic compound into which pyridoxine is partly transformed *in vivo*, is an antagonist of isoniazid's activity against tubercle bacilli *in vitro*. This observation has been confirmed in this laboratory. It should be emphasized, however, that pyridoxine and another derivative, pyradoxamine, are completely inactive (at 10 γ

[3] The courtesy of Mr. Robley S. Brannon of E. R. Squibb & Sons in supplying the pyridoxine hydrochloride employed in these studies is hereby gratefully acknowledged.

per ml.) and pyridoxal is only very weakly active in this respect. For example, at least 5 γ of pyridoxal per ml. of medium are required for detectable (twofold) antagonistic activity. Furthermore, the administration of pyridoxine in large amount (visibly coated onto food pellets) to tuberculous guinea pigs does not interfere with the chemotherapeutic activity of isoniazid given at the low daily dosage of 2.5 mg. per kg. Finally, no clinical or microbiologic evidence has been encountered in tuberculous patients treated with isoniazid at approximately 8 mg. per kg. per day plus pyridoxine hydrochloride, 25 mg. per day, that the added pyridoxine interferes in any way with the chemotherapeutic activity of isoniazid in human beings. Pyridoxine deficiency, induced in human beings by the administration of the antagonist, desoxypyridoxine, has been seen to be accompanied by peripheral neuritis (6).

It seems possible, but by no means proved in the writers' experience, that the administration of pyridoxine concomitantly with isoniazid from the time of initiation of isoniazid therapy may decrease the incidence of peripheral neuritis in human beings treated with this chemotherapeutic agent.[4] In particular, this practice may be recommended in patients who might have histories of alcoholism or of malnutrition due to other causes.

SUMMARY

Eight cases of peripheral neuritis, associated with the administration of isoniazid without vitamin supplements of the B-complex group, are described. Seven of the patients had histories of chronic alcoholism, and one suffered from pneumatosis cystoides intestinalis. On discontinuation of isoniazid and the administration of pyridoxine, only one of these patients showed progression of the neuritis, and 6 showed marked improvement within a period of a few months. Two patients with signs and symptoms of peripheral neuritis and histories of chronic alcoholism have been treated for at least six months with isoniazid (8 mg. per kg. per day, by mouth) and pyridoxine (25 mg. per day, by mouth) without progression of previously acquired neuritis.

SUMARIO

Neuritis Periférica en Tuberculosos Tratados con Isoniacida

Los 8 casos aquí descritos son de neuritis periférica enlazada con la administración de isoniacida sin complementos vitamínicos del grupo del complejo B. Siete de los enfermos tenían antecedentes de alcoholismo crónico y uno padecía de neumatosis quistoides intestinal. Al discontinuarse la isoniacida y administrarse piridoxina, uno solo de los enfermos deterioró y 6 manifestaron notable mejoría en un período de pocos meses. Dos sujetos con signos y síntomas de neuritis periférica e historias de alcoholismo crónico han sido tratados con isoniacida (8 mg. diarios por kg. oralmente) a lo menos seis meses y con piridoxina (25 mg. diarios oralmente) sin agravación de la neuritis adquirida anteriormente.

RESUME

Névrite périphérique chez des tuberculeux traités par l'isoniazide

Description de huit cas de névrite périphérique associés à l'administration d'isoniazide sans adjonction de vitamines supplémentaires du complexe vitaminique B. Parmi ces mala-

[4] During the course of the studies described here, Biehl and Vilter observed independently, and reported at the Veterans Administration, Army, Navy Conference on the Chemotherapy of Tuberculosis in St. Louis in February, 1954, that pyridoxine prevents the development of peripheral neuritis in tuberculous patients treated with isoniazid; publication of their observations (7) appeared during the preparation of this manuscript.

A detailed report on the studies by Biehl and his associates appears in this number of the REVIEW, page 430.

des, sept avaient des antécédents d'alcoolisme chronique et un était atteint de pneumatose intestinale. Lorsque l'isoniazide a été interrompu et que la pyridoxine a été administrée, un seul de ces malades a présenté une aggravation et 6 ont montré une amélioration marquée dans l'espace de quelques mois. Deux malades avec antécédents d'alcoolisme chronique et qui présentaient des symptômes objectifs et subjectifs de névrite périphérique ont été traités par l'isoniazide (8 mg par kg par jour par voie buccale) pendant au moins six mois et par la pyridoxine (25 mg par jour par voie buccale) sans aggravation de la névrite antérieurement acquise.

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MILIARY TUBERCULOSIS, TUBERCULOUS MENINGITIS, AND CHRONIC MYELOGENOUS LEUKEMIA[1]

Case Report with Autopsy Findings

EMIL ROTHSTEIN[2] AND THOMAS JARROLD

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Since the introduction of streptomycin therapy, miliary tuberculosis and tuberculous meningitis are no longer invariably fatal. While the possibility of late relapse makes the use of the word "cure" inadvisable, a number of patients have survived for five years or longer.

This report deals with a man who had had well-established myelogenous leukemia for three years when he developed miliary tuberculosis in January, 1951, and tuberculous meningitis six weeks later. With chemotherapy consisting of streptomycin and para-aminosalicylic acid (PAS), the tuberculosis became clinically inactive. The patient died almost two years later of peritonitis due to splenic abscesses and perforated gastric ulcers, possibly secondary to tuberculosis of the spleen.

CASE REPORT

E. R., a 36-year-old clothes presser, was first admitted to the Veterans Administration Center, Dayton, Ohio, on April 4, 1950. In March, 1948, he manifested symptoms of weakness and malaise. Hospitalization in a private institution led to the findings of a severe anemia. Myelocytes and myeloblasts were present in the peripheral blood, and the patient was found to have an enlarged spleen and a bone marrow typical of chronic myelogenous leukemia. He was discharged after two blood transfusions and remained in apparent good health until March, 1950, when he again experienced weakness, with pallor, headache, and cough. Upon admission to this hospital, he had fever varying from 100° to 101°F., with enlargement of the liver and spleen and of an axillary lymph node. The total leukocyte count and platelet count were normal; the erythrocyte count was 3.5 million per cu. mm., with a hemoglobin of 10 gm. per 100 ml. A differential cell count of the peripheral blood included 2 per cent myeloblasts, 5 per cent myelocytes, and 8 nucleated erythrocytes per 100 leukocytes (table 1). A sternal bone marrow aspiration revealed an increase in myelocytes and myeloblasts with a great increase in the myelo-erythroid ratio (table 2). This hematologic picture was interpreted as characteristic of chronic myelogenous leukemia. Treatment instituted shortly after admission consisted of multiple whole-blood transfusions and four X-ray treatments directed over the enlarged spleen.

A chest roentgenogram made on April 6, 1950, revealed a shadow compatible with enlarged lymph nodes in the region of the right hilum and an area of increased density in the right upper lobe compatible with a recent infiltrate due to pulmonary tuberculosis. A number of sputum examinations were negative for tubercle bacilli at this time. A biopsy of the enlarged lymph node in the left axilla was performed on April 24, 1950, and the pathologist reported, "the central section appears to be necrotic, with caseation and degenerated polymorphonuclear leukocytes and cellular debris, surrounded by an epithelioid reaction; other areas reveal dense infiltration by large reticulo-endothelial cells and stem cells."

The impression was formed that this microscopic picture was compatible with myelogenous leukemia with superimposed infectious granuloma. The etiology of the latter was clarified when a culture from the same lymph node revealed *Mycobacterium tuberculosis*.

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TABLE 1

REPRESENTATIVE TOTAL AND DIFFERENTIAL LEUKOCYTE COUNTS IN PERIPHERAL BLOOD

	SEPTEMBER 20, 1948	JANUARY 11, 1951	MARCH 1, 1951	JULY 26, 1951	FEBRUARY 12, 1952	JULY 9, 1952
Leukocytes (thousands)	11.9	6.0	1.1	2.3	66.8	8.3
Neutrophils	60	59	50	48	71	36
Metamyelocytes	5	8	2	14	0	12
Myelocytes	8	5	4	1	2	2
Myeloblasts	4	1	0	0	20	25
Lymphocytes	20	21	42	30	2	21
Monocytes	1	6	2	2	0	3
Eosinophils	2	0	0	0	0	0
Basophils	0	0	0	0	2	0
Nucleated erythrocytes per 100 leukocytes	12	5	0	0	3	5

TABLE 2

REPRESENTATIVE DIFFERENTIAL CELL COUNT IN BONE MARROW ASPIRATIONS

	NORMAL PER CENT	APRIL 5, 1950	JULY 12, 1950	JANUARY 11, 1951	MARCH 15, 1951	OCTOBER 9, 1951	DECEMBER 12, 1951	JUNE 25, 1952
Neutrophils	7–30	16.0	2.0	20.0	16.0	10.0	8.0	10.0
Metamyelocytes	13–32	17.5	23.5	12.5	21.0	23.5	18.0	13.5
Myelocytes	5–19	35.0	30.0	43.0	38.0	35.0	39.0	37.0
Myeloblasts	0.3–5	7.0	19.5	9.5	13.5	7.0	12.0	21.0
Lymphocytes	3–17	12.0	10.0	9.0	5.5	9.0	4.0	6.0
Erythroblasts	0–3	0.0	2.0	6.0	2.0	5.0	0.0	6.0
Normoblasts	20–60	10.0	18.0	4.0	12.0	13.5	4.0	7.0

The operative wound failed to heal and, because of the purulent drainage, the persistent pulmonary infiltrate, and the continuing fever, streptomycin (without PAS) was given for three months, the dosage being 1 gm. daily intramuscularly. The temperature became normal, the draining sinus healed, and the pulmonary lesion improved. The patient was discharged on September 1, 1950. He was followed on an outpatient basis; the peripheral blood and marrow continued to show typical changes of leukemia, and the right upper lobe pulmonary infiltrate persisted with enlarged nodes in the right hilum.

On January 1, 1951, the patient developed fever and dyspnea and was admitted for the second time on January 2, 1951. At this time the hemoglobin was 8 gm. per 100 ml., the erythrocyte count was 3.0 million per cu. mm., and the leukocyte count was 4,800 per cu. mm. The peripheral blood differential cell count included 5 per cent myelocytes and 1 per cent myeloblasts. The chest roentgenogram now revealed evidence of a widespread bilateral nodular infiltrative process typical of hematogenous (miliary) tuberculosis (figure 1). A culture of the sputum obtained on January 9, 1951, was positive for *M. tuberculosis*. A cerebrospinal fluid examination, including guinea pig inoculation, was normal. Treatment with streptomycin (1 gm. daily intramuscularly) and PAS (12 gm. daily orally) was begun. The daily temperature, which had been elevated to 103°F., dropped sharply three days after the start of streptomycin treatment, remaining below 100°F. until February 27, 1951, when it again ascended to 103°F.

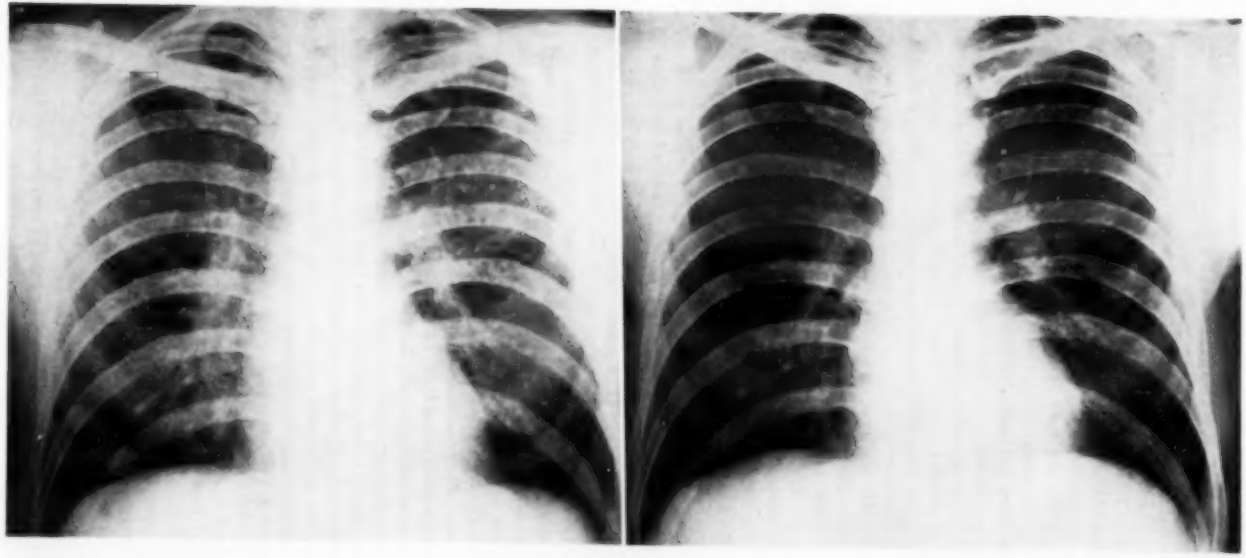

FIG. 1. (Left) Chest roentgenogram obtained on January 3, 1951. There is a bilateral symmetrical widely distributed nodular lesion, not seen on a film made three months earlier. This roentgenographic picture was associated with high fever and sputum cultures positive for *M. tuberculosis*.

FIG. 2. (Right) Roentgenogram obtained on December 20, 1951. Marked bilateral clearing of the lesions can be seen, but residual deposits persist.

TABLE 3

REPRESENTATIVE CEREBROSPINAL FLUID FINDINGS

DATE	CELLS	DIFFERENTIAL	CULTURE	GLUCOSE	PROTEIN	CHLORIDES
January 12, 1951	3		0		24 mg.	660 mg.
February 28, 1951	78	100 per cent lymphocytes	0	60 mg.	82 mg.	660 mg.
March 2, 1951	30	100 per cent lymphocytes	+ tuberculosis	42		
March 5, 1951	39	100 per cent lymphocytes	+ tuberlosis	28		
May 4, 1951	42	90 per cent lymphocytes	0	43	302 mg.	
July 20, 1951	15	100 per cent lymphocytes	0	36	149 mg.	678 mg.
October 19, 1951	11	100 per cent lymphocytes	0	38	110	660
December 7, 1951	15	100 per cent lymphocytes	0	56	84	693
March 5, 1952	2	100 per cent lymphocytes	0	51	48	643
May 14, 1952	4	100 per cent lymphocytes	0	72	58	697
October 3, 1952	2	100 per cent lymphocytes	0	78	38	702

Severe headache and mental confusion were now noted. Cerebrospinal fluid examination on February 28, 1951, revealed 89 lymphocytes per cu. mm., with elevated protein. Cultures of the cerebrospinal fluid were positive for *M. tuberculosis* on specimens obtained on March 2 and March 5, 1951. Intrathecal streptomycin was added to the therapy, 100 mg. being given daily for five days. This regimen was followed by the same dosage three times weekly for one month, twice weekly for one month, and once weekly thereafter for a total period of fifteen months. The daily intramuscular streptomycin was decreased to twice weekly after four months of daily treatment. Oral PAS, 12 gm. daily, was continued for a total period of one year. The clinical response was satisfactory. Fever disappeared as did all other evidence of toxicity. The roentgenograms showed slowly progressive clearing, although a persistent infiltrate could still be visualized one year after the start of treatment (figure 2). No further sputum or gastric cultures yielded tubercle bacilli. Cerebrospinal fluid cultures also were persistently negative, but the protein and cell counts remained slightly elevated (table 3). A streptomycin-susceptibility test performed upon the organisms from the cerebrospinal fluid obtained on March 2, 1951 (after a total of four and one-half months of streptomycin therapy), showed abundant growth (4-plus) in a control tube containing no streptomycin and in one containing 1 γ of streptomycin per ml. Slight growth was present in medium containing 10 γ per ml. and no growth in 100 γ per ml. Complete inhibition was obtained by 1 γ of PAS per ml.

A second biopsy was performed on two adjoining axillary lymph nodes on November 7, 1951. The microscopic report read, "Almost the entire central sections of both nodes are destroyed by caseation necrosis leaving only a thin rim of fibrous connective and lymphoid tissue. There are no Langhans' giant cells evident. In the lymphoid tissue there are numerous large immature cells apparently of the myeloid series. There are also numerous megakaryocytes and mature myeloid elements including eosinophils. The changes are consistent with chronic myeloid leukemia and caseous tuberculosis of the lymph nodes."

The progress of the leukemia is tabulated in table 1. It is of special interest to note that in March, 1951, the leukocyte count was below 1,000 per cu. mm. on several occasions (March 14, 1951—850; March 27, 1951—650), and severe leukopenia persisted for the first six months of his miliary tuberculosis and meningitis. No correlation could be discerned between the clinical course and the leukocyte counts. During the period of leukopenia, the platelets were increased above normal and the peripheral blood always showed 3 to 5 per cent myelocytes. The marrow remained myeloblastic. A striking decrease was noted in the size of the spleen during the period of treatment. The erythrocyte count rose to 4.0 million per cu. mm., with hemoglobin of 11.7 gm. per 100 ml.

The patient was discharged from the hospital on December 15, 1951, as an arrested, or inactive, case of miliary tuberculosis and tuberculous meningitis. Treatment was continued on an outpatient basis. This consisted of streptomycin, 1 gm. intramuscularly and 100 mg. intrathecally, given weekly. In addition, because of an increasingly elevated leukocyte count (25,000), which made its appearance after his discharge, with an increasing myeloblast percentage (15 per cent), he received urethane, 1 gm. three times daily. PAS was not given as it was not known whether urethane and PAS could safely be administered concomitantly.

On February 12, 1952, the patient was readmitted to the hospital with acute intestinal obstruction. On February 20, 1952, laparotomy was performed and revealed complete obstruction of several feet of small intestine due to adhesions. The adhesions were severed and one was biopsied. The pathologist's report read "blood clot containing numerous immature myeloid cells and undergoing early organization." Recovery from this acute abdominal emergency was slow but otherwise uneventful. Chest roentgenograms showed no further changes. The cerebrospinal fluid was examined first weekly and later at semimonthly intervals. The most recent examinations were quite normal but for an occasional slight elevation of the protein (table 3).

The leukocytic response in the peripheral blood reached a peak of 67,000 leukocytes (February 12, 1952) with 20 per cent myeloblasts and then gradually receded (tables 1 and 2).

The patient was discharged for the second time on May 8, 1952. At this point, he was in excellent general health and weighed 142 pounds (more than at any time in the previous few years). At the time of discharge, the intrathecal streptomycin was discontinued (after fifteen months of therapy). Intramuscular streptomycin (1 gm. weekly) was continued with monthly examinations of spinal taps and bimonthly chest roentgenograms. On July 1, 1952, isoniazid (150 mg. daily) was started in conjunction with the weekly streptomycin.

The patient remained in excellent general condition until shortly before his last admission to the hospital on September 18, 1952, when he developed severe chills and high fever. Upper abdominal pain was present. Blood counts revealed severe anemia (hemoglobin 5.5 gm.) and leukopenia (1,350 per cu. mm.) with essentially normal differential counts. He was treated with penicillin and with repeated blood transfusions. The course was stormy with minor remissions and relapses and was interpreted as being due to an exacerbation of his leukemia. Abdominal pain gradually increased, with distention and intermittent bouts of paralytic ileus. He was never well enough to undergo abdominal surgery, and he finally died on November 12, 1952.

Post-Mortem Examination

Post-mortem examination (Dr. A. S. Thompson, chief pathologist) was performed a few hours after death. It revealed that the immediate cause of death was a large localized peritoneal abscess, associated with two large perforated gastric ulcers and multiple abscesses of the spleen.

Specialized examinations of the lungs, meninges, and stomach are of interest.

Lungs: The pleural cavities were characterized by easily separated fibrous adhesions, mostly about the apices. The pleural surfaces were generally smooth but innumerable l

mm. whitish subpleural nodules could be seen. Cut section of the lungs again revealed "a multitude of varying-sized, discrete, whitish and cream-colored nodules, a mm. or so in diameter." These were more numerous in the lower lobes. Microscopically, these all showed homogeneous caseation necrosis surrounded by zones of partially hyalinized fibrous tissue. Epithelioid tubercles, giant cells, and tuberculous granulation tissue were lacking.

Meninges: The meninges were essentially normal. Grossly, a few scattered dural and arachnoidal adhesions were found concentrated for the most part over the cerebrum. The basal portions of the meninges were normal, as was the brain upon section. Microscopically, no evidence of tuberculosis was discovered "and nothing to suggest old or recent meningitis could be seen."

Stomach: The stomach was adherent to the spleen and to the left lobe of the liver. Dissection revealed an abscess cavity whose walls were composed of the liver, stomach, spleen, and posterior peritoneum overlying the pancreas. The abscess cavity was in continuity with large necrotic cavities extending into the spleen. Two large perforations (4.0 cm. and 2.5 cm. in diameter, respectively) were found in the posterior gastric wall. The spleen was markedly enlarged and formed a portion of the wall of the peritoneal abscess. Multiple splenic abscess cavities were found, the largest measuring 14 by 8 cm. Numerous splenic abscesses were found, not communicating with the peritoneal space, and approximately twenty-five to thirty 0.5 to 1 cm. caseous nodules.

Upon microscopic examination, there were large zones of necrosis within the spleen, with central areas of amorphous debris and a peripheral zone of nuclear fragments and cellular debris. A few zones of epithelioid cells were discovered. In addition, a diffuse infiltration with immature myeloblastic and myelocytic elements was seen. The gastric ulcers were characterized by zones of necrosis and nonspecific acute and chronic inflammatory reaction. The bone marrow revealed characteristic myelogenous leukemic changes.

The pathologist commented: "The unusual feature is the apparently normal meninges other than scattered dura-arachnoidal adhesions. The meninges were examined before and after fixation and nothing to suggest old or recent meningitis could be seen. . . .There is sufficient evidence . . . . that this is myelogenous leukemia . . . consistent with a subacute and acute phase."

Some question exists whether the splenic tuberculosis perforated into the peritoneal cavity, causing a localized tuberculous peritonitis with secondary gastric involvement, or whether the gastric ulcer was the cause of the peritonitis with secondary involvement of the spleen.

DISCUSSION

This patient with known myelogenous leukemia of three years' duration developed miliary tuberculosis and tuberculous meningitis. The tubercle bacilli in the cerebrospinal fluid were susceptible to streptomycin despite four and one-half months of prior therapy, and the patient showed an excellent response to combined chemotherapy.

Smith (1) analyzed 566 cases of miliary tuberculosis and tuberculous meningitis. From the results in this group, an estimated survival curve was prepared for cases of miliary tuberculosis alone, tuberculous meningitis alone, both diseases occurring together, and tuberculous meningitis developing in the course of miliary tuberculosis. The patient of the present report fell into the last of these categories. For this group, of a theoretical one hundred cases at the start of treatment, Smith found that thirteen might be expected to survive for one year if treated with streptomycin alone and 29 if streptomycin and PAS were used. It is possible that the earlier and poorer results in this type of case, using streptomycin alone, were due in part to the development of streptomycin-resistant tubercle bacilli. This limiting factor for long-term chemotherapy has been to a large degree eliminated in pulmonary tuberculosis by the addition of daily doses of PAS to streptomycin therapy, and available statistics indicate that a similar effect may be anticipated

in other forms of tuberculous infection. It has been recommended (2) that miliary tuberculosis and tuberculous meningitis be treated at first with daily intramuscular and intrathecal injections of streptomycin and daily oral doses of PAS and isoniazid and that, as the patient improves, the intervals of intrathecal and intramuscular injections be lengthened. During the last six months of this patient's hospitalization, the streptomycin was given intrathecally at weekly intervals and intramuscularly twice weekly, a regimen proved to be effective in pulmonary tuberculosis. For the second year of therapy, it was planned to give streptomycin once weekly (3) intramuscularly, with isoniazid, 150 mg. daily orally. In view of the fact that relapses after two years are infrequent, it was planned to continue therapy for at least this length of time.

As far as could be determined, the reported patient was the first to have a combination of leukemia, miliary tuberculosis, and tuberculous meningitis with a survival period of this length of time.

There are many interesting correlations between leukemia and tuberculosis. The nature of the relationship between these two diseases has been controversial for many years (4, 5, 6, 7, 11). Not infrequently, tuberculosis, especially the miliary type, has been associated with abnormal lymphocytic and myelocytic blood reactions resembling leukemia. When this type of cellular reaction has occurred in patients with miliary tuberculosis, opinion has been divided as to the true hematologic diagnosis. Some investigators (4, 8) believe that miliary tuberculosis and leukemia are two distinct diseases, which upon occasion may be found in the same patient. Others (9, 10), however, believe that when the two diseases are thought to coexist, the leukemia may be only apparent and the abnormal blood picture often represents, in fact, a leukemoid reaction occurring in response to miliary tuberculosis. This latter viewpoint has apparently arisen from the experience of clinicians who have followed patients with chronic leukemia who subsequently developed miliary tuberculosis. Although these patients had apparently proved leukemia of either the myelocytic or lymphocytic types, autopsy findings not infrequently failed to confirm this diagnosis. Because of the complete absence of tissue infiltration by immature cells, most of these cases were diagnosed as leukemoid reaction and not true leukemia. Still other writers (8, 12), however, explained the absence of leukemic infiltration at autopsy in these patients as being due to the effect of tuberculosis on the pre-existing leukemia. These investigators present the hypothesis that tuberculous infection through some unknown mechanism alters the course of chronic leukemia, and a reversion of the hematologic system towards normal follows.

In the present case, it was believed that the clinical diagnosis of chronic myelogenous leukemia had been established beyond doubt. The characteristic bone marrow and peripheral blood findings antedated by approximately two years the appearance of tuberculosis. A marked leukopenia developed concomitantly with the appearance of miliary tuberculosis, but immature cells were always found in the peripheral blood and "blastic" bone marrows were present throughout the entire period of study. In addition, a lymph node biopsy taken before the appearance of miliary tuberculosis and again approximately one year after its onset revealed a microscopic picture quite compatible with leukemia as well as miliary tuberculosis. The post-mortem examination confirmed this opinion.

The natural course of chronic myelogenous leukemia is quite variable and, in any individual, the prognosis is dependent upon many factors. The average life expectancy with this disease has been generally estimated to be approximately three years after the onset of symptoms. The patient of the present report died four and a half years after his leukemia had been diagnosed.

When the patient was first treated with streptomycin for the draining sinus following

a lymph node biopsy, the drug was given without PAS. This was probably done on the assumption that, as he was "incurable," one might dispense with this ancillary drug, which often produces unpleasant gastrointestinal symptoms. The subsequent course of the patient's illness illustrated the fact that one should not withhold treatment which may prolong a patient's life for months or years just because the condition is at present in the "incurable" category. Just as patients with malignant lymphoma are treated with nitrogen mustard, X-ray, and radio-isotopes in order to give them less distress during their few remaining months or years, so too should tuberculosis be treated adequately, at least by medical means, even in the presence of lymphoma or other "incurable" disorders.

Of considerable interest from the standpoint of the body's response to tuberculosis was the finding that the profound leukopenia which the patient presented during the early part of his illness apparently had no effect in preventing a satisfactory response of the tuberculosis to treatment. It may be presumed that the suppressive effect of the combined chemotherapy more than made up for any loss in cellular immunity which might be related to a marked drop in circulating leukocytes. There is no reason to believe that the tissue factors which respond to the presence of the tubercle bacillus showed a similar suppression.

SUMMARY

A patient with myelogenous leukemia of three years' duration developed miliary tuberculosis and tuberculous meningitis. After one year of streptomycin and PAS treatment, his tuberculosis became clinically arrested and, from the standpoint of the tuberculosis, the disease process remained inactive. Death was due to sepsis following perforation of the stomach and of splenic abscesses with the development of a localized peritoneal abscess. At autopsy, the meninges were normal and apparently healing miliary tuberculosis was found. The response to treatment, although temporary, suggests that active antituberculous therapy is indicated even when a patient is also ill with another disease with an eventual hopeless prognosis.

SUMARIO

Granulia, Meningitis Tuberculosa y Leucemia Mielógena Crónica: Historia Clínica con Hallazgos Autópsicos

Un enfermo que había tenido leucemia mielógena durante tres años manifestó granulia y meningitis tuberculosa. Al cabo de un año de tratamiento combinado con estreptomicina y ácido para-aminosalicílico, el caso se estacionó clínicamente y, desde el punto de vista de la tuberculosis, el proceso patológico permaneció inactivo. La muerte se debió a sepsis consecutiva a la perforación del estómago y a abscesos esplénicos con formación de un absceso peritoneal localizado. En la autopsia, las meninges se hallaban en estado normal y se observó granulia aparentemente en vías de cicatrización. La respuesta al tratamiento, aunque temporal, sugiere que está indicada la terapéutica antituberculosa activa, aun cuando un sujeto padezca además de otra dolencia cuyo pronóstico se considere a la larga desesperado.

RESUME

Tuberculose miliaire, méningite tuberculeuse et leucémie myélogène chronique. Observation d'un cas et résultats de l'autopsie

Une tuberculose miliaire et une méningite tuberculeuse se sont développées chez un malade atteint depuis trois ans de leucémie myélogène. Au bout d'un an d'un traitement par

la streptomycine et l'acide para-aminosalicylique combinés, une stabilisation clinique a été obtenue et, du point de vue tuberculose, le processus actif de la maladie a cessé. La mort a été causée par une septicémie, qui a succédé à une perforation de l'estomac, et par des abcès spléniques avec production d'un abcès péritonéal localisé. A l'autopsie, les méninges étaient normales et les lésions miliaires étaient visiblement en voie de guérison. La réponse au traitement, bien que temporaire, suggère qu'une thérapie antituberculeuse active est indiquée, même lorsqu'un malade est atteint d'une maladie dont le pronostic ultime est sans espoir.

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Editorial

Thirty Years of Tuberculosis Therapy in a Municipal Sanatorium

Therapeutic efforts in pulmonary tuberculosis have come full circle during the past thirty years. The gold preparation, sanocrysin, which was the favored chemotherapeutic agent of the early nineteen twenties, was replaced during the next ten years by collapse therapy. Today, chemotherapy with truly effective agents is back in the saddle and the use of pneumotherapy[1] has greatly decreased. The Wm. H. Maybury Sanatorium records covering this interesting period in phthisiology have been reviewed in an attempt to bring the present scene into perspective.

The initial experience with chemotherapy at Maybury was on an experimental basis only. Sanocrysin came to this country recommended as highly efficacious by certain European workers[2], an efficacy which was questioned by Dr. Max Pinner and Dr. J. Burns Amberson, then the Controller of Tuberculosis for the city of Detroit. The experimental study of the drug which they initiated at Maybury Sanatorium in December, 1926[3], sounded the death knell of sanocrysin as a therapeutic agent in pulmonary tuberculosis.

Collapse therapy, also, was introduced to the sanatorium in 1926. Its use during the following decade increased rapidly, reaching a maximum in 1937, when 47.1 per cent of all Maybury patients were treated with pneumothorax. From this maximum the use of artificial pneumothorax decreased, at first gradually, then more rapidly to a low level of 12.8 per cent in 1944, with the publication of Rafferty's book[4] on indications and contraindications for pneumothorax. In the following year a new upward trend in the total use of pneumotherapy began, for two reasons. First, the introduction of pneumoperitoneum offered a means of collapsing the lungs which seemed safer than pneumothorax. Secondly, when chemotherapy returned to the sanatorium in 1947, as streptomycin became available, it soon became evident that "resistant" tubercle bacilli appear most rapidly in streptomycin-treated patients who have open cavities[5]. During 1949 and 1950, this resulted in the administration of some form of pneumotherapy to nearly every patient who received streptomycin.

In addition to pneumotherapy, the Maybury records show that collapse of

[1] A term suggested by Dr. Julius Wilson to include both pneumothorax and pneumoperitoneum, Am. Rev. Tuberc., 1953, *68*, 954.

[2] (*a*) Møllgaard, H.: Nyt Nordisk Forlag', Copenhagen, 1924. (*b*) Secher, K.: Beitr. z. klin. d. Tuberk., 1929, *72*, 174.

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[5] (*a*) Howard, W. L., Maresh, F., Mueller, E. E., Yannitelli, S. A., and Woodruff, C. E.: Am. Rev. Tuberc., 1949, *59*, 391. (*b*) Howlett, K. S., Jr., O'Connor, J. B., Sadusk, J. F., Jr., Swift, W. E., Jr., and Beardsley, F. A.: Am. Rev. Tuberc., 1949, *59*, 402.

the lung was frequently obtained by diaphragmatic paralysis. In 1935, nearly 40 per cent of Maybury patients were subjected to surgical interruption of the phrenic nerve, more than 47 per cent in 1949.

The use of para-aminosalicylic acid (PAS) in the treatment of pulmonary tuberculosis was introduced in the United States in 1947 and, during the succeeding two years, its use became widespread. In July of 1951, long-term therapy with streptomycin-PAS became the standard at Maybury. With this development and the resulting decreased need for the artificial collapse of cavities, the proportion of Maybury patients who received pneumotherapy fell abruptly from 35.5 per cent in the first part of 1951 to 0 per cent in 1953. Also, the year 1953 saw no phrenic nerve operations, although 101 patients were referred for either thoracoplasty or excisional surgery. The complete abandonment of temporary collapse of the lung indicates an overwhelming vote of confidence in long-term chemotherapy, which is now being given to 91.5 per cent of the sanatorium patients.

Changes in the sanatorium death rate (annual deaths divided by the average daily patient census) during the past thirty years have proved interesting when compared with the type of therapy being employed. From 1928 to 1932, for example, while the use of pneumothorax was increasing most rapidly, the death rate fell from 13.5 per cent in 1929 to 3.5 per cent in 1932, giving rise to a premature feeling of optimism concerning the effectiveness of collapse therapy. This optimism was soon dissipated as the death rate began to climb again in spite of continued extensive use of pneumothorax. During the 1940 to 1945 interval, while the use of pneumothorax was being severely curtailed, the death rate rose from 19.3 to 28.8. This again does not represent a cause and effect relationship but is due to a third factor—the type of patient admitted to the sanatorium.

Actually, the records show that, during the nineteen thirties and forties, the death rate at Maybury was determined largely by the degree of illness of the newly admitted patients. Thus, in 1929, eight patients were admitted who lived for less than one month. In 1932, only two patients were in this category; in 1945, there were thirty-three. The number of these critically ill patients who came to the sanatorium is rather closely related to the total industrial effort in the city of Detroit. In 1929, for example, 5,300,000 automobiles were produced in the United States, most of them in the Detroit area.[6] In 1932, only 1,300,000 cars were produced. During the war years, from 1940 to 1945, the output of automobiles was limited by law, but the total industrial effort on war materiel was at a maximum. It is significant that the highest death rate at the sanatorium occurred in 1945. In the final period of observation, from 1951 to 1954, when pneumotherapy was completely abandoned in favor of long-term chemotherapy, the death rate fell from 19.5 in 1951 to 6.9 in 1953. At the same time industrial activity was high, as indicated by the production of 7,300,000 automobiles in 1953. The continued decline of the sanatorium death rate in the face

[6] Maybury Sanatorium, although a Detroit Municipal Institution, receives tuberculosis patients from all of Wayne County.

of this high level of industrial activity must be taken, in part at least, as evidence of the effectiveness of present-day chemotherapy.

The survey of Maybury Sanatorium records emphasizes the cyclical character of tuberculosis therapy during the past several decades. It reminds one also of the possibility that today's standard therapy will be altered or abandoned during the next ten years. Will future years see a return to the use of collapse therapy? This possibility will be discussed elsewhere.[1] For the present, suffice it to say that changes which occurred in the sanatorium death rate during the nineteen thirties and forties were more dependent upon the proportion of critically ill patients sent to the sanatorium than on the type of therapy. The decline in the death rate of the past three years, however, is believed to be due in large measure to the effectiveness of long-term antimicrobial therapy.

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[1] See page 533 of this issue of the Review.

Notes

UNITED STATES PUBLIC HEALTH SERVICE COOPERATIVE INVESTIGATION OF ANTIMICROBIAL THERAPY OF TUBERCULOSIS

V. Report on Thirty-Two-Week Observations on Combinations of Isoniazid, Streptomycin, and Para-Aminosalicylic Acid

Although isoniazid is generally recognized as a powerful drug presenting new possibilities for the attack on tuberculosis, many questions still remain concerning its therapeutic use. Two of the simpler questions, namely, the result of increasing the daily dose and the effectiveness of its combination with streptomycin and para-aminosalicylic acid (PAS), are being investigated in the second of the current Public Health Service studies. This note reports the results of thirty-two weeks of observation.

Physicians[1] in twenty-three hospitals throughout the country are treating 1,800 patients according to a protocol adopted in March, 1953. The plan of the study has been outlined in the first progress report.[2] The five drug regimens under investigation are:

Isoniazid (3 mg. per kg.) plus streptomycin
Isoniazid (3 mg. per kg.) plus PAS
Isoniazid (10 mg. per kg.) plus streptomycin
Isoniazid (10 mg. per kg.) plus PAS
Isoniazid (10 mg. per kg.) plus streptomycin and PAS

Streptomycin is given in doses of 1 gm. twice a week; PAS, 10 to 12 gm. daily; and isoniazid, 3 or 10 mg. per kg. daily.

Limiting the study to patients without previous chemotherapy so slowed the rate at which suitable cases could be found that by September, 1953, only 1,284 patients had been admitted to the study. The number of patients randomly assigned to each of the five treatment regimens ranged from 252 to 262. Although these patients could have completed thirty-two weeks of treatment by April of this year, 516 of them (40 per cent) were by that time no longer being treated according to the protocol and have been excluded from the analysis of therapeutic efficacy. Nearly 97 per cent of the excluded patients did not follow the prescribed chemotherapy for one of two reasons, departure from the hospital or drug intolerance. Of the remainder, divided among all five regimens, 10 refused, 5 had serious illness other than tuberculosis, and treatment for 3 was changed in error.

Toxicity: In the first progress report, the fact that the proportions of patients leaving the hospital were not the same for each regimen left open the possibility that the assignment of particular chemotherapeutic agents might have affected the frequency of self-discharges. However, with the observation of more patients for a longer period of time, no relation is apparent between the drugs received and the likelihood that a patient will leave the hospital. As shown in the fourth column of table 1, roughly one-fourth of the patients on each regimen had left the hospital by the thirty-second week.

The choice of drugs does, however, affect a patient's chances, while in the hospital, of remaining on the assigned chemotherapy. Drug toxicity necessitated a change in

[1] The investigators were listed in the January, 1954, issue of the REVIEW, page 1. Dr. Francis J. Murray of the central office provided liaison among the clinical investigators.

[2] Am. Rev. Tubere., 1954, *69*, 1.

TABLE 1

NUMBER AND PER CENT OF PATIENTS ON EACH REGIMEN EXCLUDED FROM ANALYSIS OF THERAPEUTIC EFFICACY BECAUSE OF FAILURE TO FOLLOW PRESCRIBED CHEMOTHERAPY

REGIMEN	NUMBER ASSIGNED	NUMBER EXCLUDED	PER CENT EXCLUDED BY REASON			
			Total	Left Hospital	Toxicity	All Other
Total	1,284	516	40.2	23.5	15.3	1.4
3 mg. of isoniazid per kg. plus streptomycin	252	80	31.8	25.4	4.4	2.0
3 mg. of isoniazid per kg. plus PAS	252	92	36.5	23.4	11.5	1.6
10 mg. of isoniazid per kg. plus streptomycin	262	95	36.2	20.2	14.5	1.5
10 mg. of isoniazid per kg. plus PAS	261	118	45.3	21.1	23.4	0.8
10 mg. of isoniazid per kg. plus streptomycin plus PAS	257	131	51.0	27.6	22.2	1.2

treatment for 196 patients, 38 of whom developed toxic symptoms to two drugs and 7 to all three drugs.

The fifth column of table 1 shows for each regimen the per cent of patients who could not tolerate one or more of the drugs received. Regimens using both PAS and 10 mg. of isoniazid have the highest rates of toxicity, approximately 23 per cent. The least toxic regimen—with a rate of only 4 per cent—was the one in which the individual drugs were least toxic, 3 mg. of isoniazid plus streptomycin.

The relative toxicity of the individual drugs is indicated by the finding that toxic symptoms developed in 4 per cent of the patients receiving 3 mg. of isoniazid, 14 per cent of those receiving 10 mg., 11 per cent of those receiving PAS, and 5 per cent of those on streptomycin.

Characteristics of case material: Among the 768 patients observed for thirty-two weeks, 172 received 3 mg. isoniazid per kg. plus streptomycin, 160 received 3 mg. isoniazid per kg. plus PAS, 167 received 10 mg. isoniazid per kg. plus streptomycin, 143 received 10 mg. isoniazid per kg. plus PAS, and 126 received 10 mg. isoniazid per kg. plus streptomycin plus PAS.

At the beginning of treatment, the five treatment groups were comparable with respect to the characteristics of the patients and their disease on which information was available. The disease in 5 per cent of all patients was minimal in extent, moderately advanced in 40 per cent, and far advanced in 55 per cent. Roentgenograms for nearly 50 per cent of the patients showed either no cavity or cavities of less than 2 cm. in diameter; for 25 per cent, cavities between 2 and 4 cm. in diameter; and for the remaining 25 per cent, cavities with a total diameter of more than 4 cm. Only 12 per cent of the patients had been hospitalized for more than three months.

Deaths: Eleven patients died of tuberculosis during the thirty-two weeks of observation: 3 who had received 10 mg. isoniazid per kg. plus streptomycin plus PAS, 3 treated with 10 mg. isoniazid per kg. plus streptomycin, 2 on each regimen combining isoniazid and PAS, and one who received 3 mg. isoniazid per kg. plus streptomycin.

Roentgenographic changes: Roentgenographic data for this report were obtained from an evaluation conference of the clinical investigators held in April, 1954. As in previous

TABLE 2

PERCENTAGE DISTRIBUTION OF ROENTGENOGRAPHIC CHANGE AT FOUR-WEEK INTERVALS IN 768 PATIENTS OBSERVED FOR THIRTY-TWO WEEKS

	CHANGE FROM BEGINNING OF TREATMENT								
	Total	Improvement			No Change	Deterioration			Death
		Marked	Moderate	Slight		Slight	Moderate	Marked	
3 Mg. of Isoniazid Per Kg. Plus Streptomycin—172 Patients									
4 weeks	100.0	0.6	6.4	41.6	50.0	1.4	—	—	—
8 weeks	100.0	2.1	21.9	46.0	27.6	1.6	0.8	—	—
12 weeks	100.0	6.5	32.9	41.3	16.3	1.4	0.2	1.4	—
16 weeks	100.0	11.9	40.7	32.7	11.9	1.2	0.2	1.4	—
20 weeks	100.0	21.1	40.7	26.0	9.8	0.4	0.4	1.6	—
24 weeks	100.0	29.3	38.6	21.4	7.5	0.6	0.6	1.4	0.6
28 weeks	100.0	35.3	37.1	18.0	6.6	1.0	—	1.4	0.6
32 weeks	100.0	41.9	34.1	14.5	6.5	0.6	0.6	1.2	0.6
3 Mg. of Isoniazid Per Kg. Plus PAS—160 Patients									
4 weeks	100.0	0.4	6.7	34.5	55.1	1.7	0.4	0.6	0.6
8 weeks	100.0	2.3	19.0	47.1	28.1	1.5	0.8	0.6	0.6
12 weeks	100.0	6.3	30.7	41.2	18.2	1.3	1.1	0.6	0.6
16 weeks	100.0	12.6	37.7	34.7	11.0	0.8	1.3	0.6	1.3
20 weeks	100.0	21.0	40.7	24.5	9.4	1.0	0.6	1.5	1.3
24 weeks	100.0	26.8	44.4	18.2	6.5	1.1	0.4	1.3	1.3
28 weeks	100.0	35.4	39.7	16.3	5.2	0.4	0.4	1.3	1.3
32 weeks	100.0	43.5	35.4	13.4	4.3	0.2	0.6	1.3	1.3
10 Mg. of Isoniazid Per Kg. Plus Streptomycin—167 Patients									
4 weeks	100.0	0.8	7.6	38.4	52.2	0.4	—	—	0.6
8 weeks	100.0	2.8	20.0	46.7	28.7	1.0	0.2	—	0.6
12 weeks	100.0	6.4	37.3	38.4	16.3	1.0	—	—	0.6
16 weeks	100.0	15.1	42.6	30.1	10.8	0.6	0.2	—	0.6
20 weeks	100.0	23.3	45.8	22.7	7.0	0.2	0.4	—	0.6
24 weeks	100.0	33.1	40.4	19.0	5.7	0.6	—	—	1.2
28 weeks	100.0	39.6	38.1	15.2	4.9	0.8	0.2	—	1.2
32 weeks	100.0	48.8	30.8	12.7	4.2	1.0	0.4	0.2	1.9
10 Mg. of Isoniazid Per Kg. Plus PAS—143 Patients									
4 weeks	100.0	—	8.6	30.5	59.3	—	0.2	0.7	0.7
8 weeks	100.0	2.4	22.6	41.5	30.7	0.5	0.2	1.4	0.7
12 weeks	100.0	6.9	33.9	37.5	19.2	0.2	0.2	1.4	0.7
16 weeks	100.0	12.0	40.6	31.7	12.9	—	—	1.4	1.4
20 weeks	100.0	19.8	42.1	25.4	9.7	0.2	—	1.4	1.4
24 weeks	100.0	31.8	39.0	18.9	7.2	0.3	—	1.4	1.4
28 weeks	100.0	39.9	37.5	12.7	6.6	0.5	—	1.4	1.4
32 weeks	100.0	45.1	36.4	10.8	4.4	0.5	—	1.4	1.4
10 Mg. of Isoniazid Per Kg. Plus Streptomycin Plus PAS—126 Patients									
4 weeks	100.0	0.8	7.4	34.6	54.8	1.1	1.3	—	—
8 weeks	100.0	3.2	23.6	43.1	28.0	0.5	0.5	0.3	0.8
12 weeks	100.0	7.5	35.5	38.1	16.3	1.3	0.5	—	0.8
16 weeks	100.0	15.9	44.9	26.3	11.0	0.8	0.3	—	0.8
20 weeks	100.0	25.0	42.8	23.4	5.6	1.1	0.5	—	1.6
24 weeks	100.0	34.3	40.4	17.0	5.6	1.1	—	—	1.6
28 weeks	100.0	41.4	38.9	11.7	5.9	0.5	—	—	1.6
32 weeks	100.0	47.4	35.2	10.9	3.8	0.3	—	—	2.4

TABLE 3

PERCENTAGE OF PATIENTS ON EACH REGIMEN IN UNFAVORABLE CLINICAL CONDITION, BY WEEK OF TREATMENT

	5 MG. OF ISONIAZID PER KG. PLUS STREPTOMYCIN	5 MG. OF ISONIAZID PER KG. PLUS PAS	10 MG. OF ISONIAZID PER KG. PLUS STREPTOMYCIN	10 MG. OF ISONIAZID PER KG. PLUS PAS	10 MG. OF ISONIAZID PER KG. PLUS STREPTOMYCIN PLUS PAS
	Number of Cases				
	172	160	167	143	126
Per Cent with Sputum Positive for Tubercle Bacilli by Direct Examination					
Selection	64.5	60.6	65.3	65.7	64.3
4 weeks	41.5	39.6	39.8	34.0	37.3
8 weeks	31.0	25.3	27.5	23.1	23.0
12 weeks	21.5	18.1	20.4	16.1	12.7
16 weeks	19.9	13.1	15.7	12.6	11.2
20 weeks	17.5	13.2	10.8	11.9	7.2
24 weeks	15.2	9.5	10.8	7.0	6.5
28 weeks	15.5	8.9	7.9	10.5	4.8
32 weeks	13.5	8.3	10.3	7.8	5.6
Per Cent with Sputum Positive for Tubercle Bacilli by Culture					
Selection	78.6	78.7	84.9	74.0	80.4
4 weeks	58.9	49.9	60.4	40.2	46.4
8 weeks	30.5	35.2	48.4	34.1	30.5
12 weeks	29.4	25.5	27.3	16.0	22.8
16 weeks	16.1	16.3	21.9	7.7	10.1
20 weeks	17.2	9.3	14.1	7.9	7.1
24 weeks	17.4	10.0	13.7	6.8	6.3
28 weeks	13.9	8.5	8.7	5.5	2.6
32 weeks	14.4	7.3	10.6	6.3	3.5
Per Cent Febrile*					
Selection	39.5	40.6	40.7	42.0	46.1
4 weeks	15.7	17.5	21.0	16.1	19.9
8 weeks	14.6	12.5	13.8	13.3	12.7
12 weeks	14.6	11.8	9.6	11.2	15.1
16 weeks	11.1	9.4	9.6	7.0	11.9
20 weeks	8.9	8.9	10.2	8.4	15.1
24 weeks	11.8	10.8	6.6	6.3	10.4
28 weeks	10.8	7.1	10.9	7.9	7.5
32 weeks	4.3	5.8	6.7	7.3	10.1
Per Cent Underweight†					
Selection	62.2	63.8	62.7	73.5	60.3
4 weeks	52.9	53.7	50.0	60.9	48.0
8 weeks	44.4	49.3	40.6	54.9	44.4
12 weeks	39.5	42.8	33.5	52.1	41.3
16 weeks	37.7	39.4	30.9	44.8	36.5
20 weeks	36.6	40.0	29.0	43.4	32.5
24 weeks	33.8	38.0	26.4	40.1	28.8
28 weeks	33.7	38.0	27.0	38.5	27.2
32 weeks	33.4	34.2	27.6	39.0	29.3

* Median of highest oral temperature for five days: febrile 99.0°F. and over.

† Minus deviation of more than 10 per cent from standard height-weight values.

conferences, films for each patient were judged independently by three physicians without knowledge of the patient's identity, clinical condition, or treatment. The percentage distribution of the resulting opinions of roentgenographic change between selection and each subsequent four-week period is presented in table 2 for each regimen.

Throughout observation, roentgenographic improvement appeared at approximately the same rate in the five treatment groups. By the thirty-second week, the proportion of films showing at least slight improvement varied only between 91 and 94 per cent among the regimens. When restricted to significant (marked or moderate) improvement, the percentages ranged between 76 and 83 per cent.

Bacteriologic and clinical changes: In table 3 are summarized the changes in sputum, fever, and body weight, and the percentage of patients on each regimen whose condition was unfavorable at each four-week period is shown.

Sputum specimens were examined for *M. tuberculosis* both directly and by culture. At the beginning of the study, tubercle bacilli were observed in sputum specimens from slightly less than two-thirds of the patients by microscopy and from slightly more than three-fourths by culture. The decrease, whether measured by direct examination or by culture, is similar for all regimens during the first twelve weeks of treatment. There is some indication that after the twelfth week the regimens which included PAS showed a more rapid decrease in the percentage of patients continuing to produce sputum positive for tubercle bacilli.

As the clinical investigators were free to perform surgery on any patient during the treatment period, the question has arisen of the extent to which reversal of infectiousness resulted from surgical procedures. During the thirty-two weeks of observation, 54 (7 per cent) of the 768 patients had surgery: 50 had some form of resection, and the remaining 4 had thoracoplasties. The number of surgical cases was approximately equal in the five regimens. Forty-six of the patients had had sputum cultures negative for tubercle bacilli for at least twelve weeks before surgery, 3 for eight weeks, and one for four weeks. Of the 4 with positive cultures at the time of surgery, 2 became negative immediately after and 2 remained positive for tubercle bacilli. Apparently, these surgical cases represent almost entirely those who have reached a so-called "target point"—not those for whom surgery was performed in an effort to reverse their infectiousness.

At the beginning of treatment, approximately 40 per cent of the patients had temperatures above 99.0°F. This proportion decreased rapidly on all regimens to 20 per cent by the fourth week of treatment. Further reduction was slow, so that by the sixteenth week approximately 10 per cent of the patients were febrile, and there was little change during the remaining period of observation.

Patients more than 10 per cent below a so-called "normal" weight according to a standard height-weight chart have been classified as underweight. At the beginning of treatment, slightly more than 60 per cent of the patients were underweight except in the group receiving 10 mg. isoniazid per kg. plus PAS, in which 75 per cent were underweight. Gain in weight during treatment was about equal on all regimens so that, by twenty-four weeks, roughly one-third remained underweight with little further change by thirty-two weeks.

In summary: This note reports therapeutic and toxic effects observed in a study comparing the usefulness of two dosage levels of isoniazid and of various combinations of isoniazid with streptomycin and PAS. From 1,800 patients with fairly recent disease without previous antimicrobial therapy, the present progress report deals with 1,284 who could have been observed at least thirty-two weeks. The heavy losses among the 1,284, due principally to self-discharges and toxicity, reduced to 768 the number who remained

under treatment for thirty-two weeks. From the information available, no disturbance could be detected in the comparability of the five treatment groups achieved by the initial random assignment. Nearly one-seventh of the patients who received a daily dose of 10 mg. of isoniazid per kg. of body weight could not tolerate the drug at that dose, although very little toxicity appeared with the 3 mg. per kg. dose. Moreover, the regimens employing the lower dose of isoniazid produced roentgenographic clearing and symptomatic improvement as frequently as the higher dose.

Comparison of the results achieved with isoniazid in various combinations with the other agents gives no clear indication that any combination is superior. The choice between streptomycin and PAS as partners for isoniazid must be made on other grounds than therapeutic effectiveness. Although streptomycin is a much more powerful agent than PAS, one is as effective as the other in combination with isoniazid. If the current rejection of isoniazid alone persists, then the lesser risk in combined therapy may be to use PAS with isoniazid and to reserve streptomycin for later use.

In the present series of cases, the combination of all three drugs for nearly eight months of treatment showed no advantage over the pairing of only two. Although this study provides no justification for the simultaneous use of isoniazid, streptomycin, and PAS in previously untreated cases of recent origin, this deliberate selection of cases may have excluded from investigation the very patients for whom three drugs might be more effective. The possibility is suggested by the high percentage of each group showing roentgenographic improvement during treatment: when most of the patients improve on two drugs, small opportunity remains for three drugs to demonstrate any superiority. A test of the three-drug combination might better be carried out on patients of whom a sizable proportion do not improve on two drugs.

Division of Special Health Services
Public Health Service
Department of Health, Education, and Welfare
Washington, D. C.

Frank W. Mount
Shirley H. Ferebee

June 14, 1954

THE IMMUNIZING PROPERTIES OF AN ISONIAZID-RESISTANT MUTANT OF THE VALLÉE STRAIN OF M. TUBERCULOSIS AS COMPARED WITH BCG[1]

Observations in the Mouse and Guinea Pig

The possibility that isoniazid-resistant mutants of strains of *M. tuberculosis* might be capable of immunizing or protecting animals against experimental tuberculous infection was first suggested by Duca and his associates.[2] The present study was undertaken to evaluate this possibility.

Method: The Vallée bovine strain of *M. tuberculosis* was used as the infecting agent throughout. An isoniazid-resistant mutant of this strain, requiring approximately 63 γ of isoniazid per ml. of liquid Tween®-albumin medium for inhibition of growth, in most instances served as the "immunizing agent." In a few instances, the Phipps strain of BCG was used for comparison.

All animals were initially injected by the subcutaneous route with a single dose of 2.2 to 10 million viable cell units of either the isoniazid-resistant strain or the BCG strain. Mice were challenged by the intravenous route fourteen to twenty-one days later, while guinea pigs were challenged subcutaneously after demonstration that the tuberculin reaction in all animals had converted from negative to positive following injection of the "immunizing agent." Three to 25 million viable cell units of the parent susceptible Vallée strain were used as the challenge dose.

Results were interpreted in guinea pigs on the basis of the extent of disease as observed grossly and microscopically at the time of death or when sacrificed. In mice, results were interpreted on the basis of per cent survival and average survival time and on the basis of histopathologic examination of tissues at the time of death.

The results obtained in representative experiments are reported herein.

Observations in guinea pigs: Figure 1 illustrates graphically the results obtained in guinea pigs, as evidenced by gross and microscopic observations. It is apparent that the isoniazid-resistant mutant of the Vallée strain of *M. tuberculosis* possesses virtually no virulence for this animal species. Only 1 of 15 animals showed evidence of tuberculous infection after receiving 2.2 million viable cell units subcutaneously. In this single animal the foci were few in number and limited to the lung and spleen. All guinea pigs infected with the parent isoniazid-susceptible strain, on the other hand, showed evidence of overwhelming disease in the lungs, liver, and spleen. Animals injected with the resistant mutant and then challenged subsequently with the susceptible virulent strain showed overwhelming disease in only 2 and moderately extensive disease in only 3 of 15 animals. Of the remaining 10 animals in the series, one showed slight disease, 3 showed sparse lesions in one organ only, and 6 showed no evidence of tuberculous disease or of healed or healing tuberculous lesions.

BCG, like the isoniazid-resistant mutant of the Vallée strain, was incapable of producing progressive tuberculous disease in the guinea pig. When administered 28 days prior to challenge with the virulent Vallée strain, overwhelming tuberculous infection resulted in 4 of 15 animals only, while moderately extensive disease was noted in 6, slight disease in 4, and sparse lesions in one.

[1] Presented in part at the Thirteenth Conference on the Chemotherapy of Tuberculosis, held under the auspices of the Veterans Administration, Army, and Navy, February 8–11, 1954.

[2] Duca, C., and Mast, G.: Personal communication.

IMMUNIZED WITH INH-R MUTANT (VALLÉE) UNINFECTED
IMMUNIZED WITH INH-R MUTANT (VALLÉE) INJECTED WITH PARENT VALLÉE STRAIN
IMMUNIZED WITH BCG (PHIPPS) UNINFECTED
IMMUNIZED WITH BCG (PHIPPS) INJECTED WITH PARENT VALLÉE STRAIN
NON-IMMUNIZED INFECTED WITH PARENT VALLÉE STRAIN

FIG. 1. Immunization against tuberculous infection in guinea pigs, due to the Vallée strain of *M. tuberculosis*: 2.2 million viable cell units of an isoniazid-resistant (INH-R) mutant of the Vallée strain or 2.7 million viable cell units of BCG (Phipps) were injected subcutaneously 28 days prior to subcutaneous infection with 4.9 million viable cell units of the parent isoniazid-susceptible Vallée strain.

It would appear that the isoniazid-resistant strain used in this study is as effective as BCG as an "immunizing agent" against infection of guinea pigs with the Vallée strain of virulent bovine tubercle bacilli.

Observations in mice: As in guinea pigs, the isoniazid-resistant mutant of the Vallée strain possesses no virulence for mice when injected either subcutaneously or intravenously. In like manner the susceptible parent strain shows no virulence for mice when injected subcutaneously.

Intravenous injection of the susceptible parent strain results in extensive tuberculous infection of all animals. As illustrated in table 1, the per cent survival and the average survival time at 28 days after infection with this virulent strain was significantly less than in animals first injected subcutaneously with the avirulent resistant mutant and subsequently challenged intravenously with the virulent susceptible strain. Although a significant difference was noted between the average survival time of immunized and nonimmunized animals as long as 50 to 77 days after intravenous infection with the virulent strain, complete protection was never achieved in any immunized series. Histopathologic examination of the tissues from these animals has offered confirmation of the fact that immunization with an attenuated isoniazid-resistant mutant of the Vallée strain, although producing adequate immunity to prolong survival time, failed to prevent ultimate development of the disease.

It is of interest that the isoniazid-resistant strain utilized in these experiments was not composed entirely of resistant cells. Approximately 95 per cent of the cells in the

TABLE 1

IMMUNIZATION WITH AN ISONIAZID-RESISTANT MUTANT OF THE VALLÉE STRAIN OF M. TUBERCULOSIS IN MICE

EXPERIMENT NUMBER	M. TUBERCULOSIS (VALLÉE)*		SURVIVAL							
			Per cent				Average Number of Days			
	Immunizing Agent (Subcutaneous Route)	Infecting Agent (Intravenous Route)	Days of Observation							
			28	35	50	77	28	35	50	77
1.	Isoniazid-resistant	—	100	100	95	95	28	35	50	75
	Isoniazid-resistant	Isoniazid susceptible	95	66	6	0	28	34	38	40
	—	Isoniazid-susceptible	30	15	0	0	26	27	28	28
	—	Isoniazid-resistant	100	100	100	100	28	35	50	77
2.	Isoniazid-resistant	—	100	100	100		28	35	50	
	Isoniazid resistant	Isoniazid-susceptible	95	55	35		28	34	40	
	—	Isoniazid-susceptible	35	15	15		24	26	28	
	—	Isoniazid-resistant	100	100	100		28	35	50	
	Isoniazid-susceptible	—	100	100	100		28	35	50	

* The number of viable cell units of the isoniazid-resistant strain injected, either subcutaneously or intravenously, ranged from 1 to 3 million in experiment number 1, and from 4 to 7 million in experiment number 2. The number of viable cell units of the parent susceptible strain injected was 13 million in experiment number 1, and ranged from 3 to 5 million in experiment number 2. There was a 21-day interval between the injection of the immunizing and infecting agents.

i

mmunizing dose required less than 10, although more than 1, γ of isoniazid per ml. of medium for inhibition of growth. The remaining 5 per cent were resistant to 100 γ of isoniazid per ml. Preliminary studies to determine the degree of immunization produced by this strain in animals treated with isoniazid for periods of time adequate to eliminate susceptible cells from the animal body have indicated a slight increase in the per cent survival and in the average survival time over that observed in untreated animals. Studies are in progress to evaluate the significance of this preliminary observation.

Comment: The diminished virulence of isoniazid-resistant strains of *M. tuberculosis* for guinea pigs has been reported previously by Steenken and Wolinsky,[3] and by others.[4,5] Although Reitman and Iverson[6] have reported isolation of a dihydrostreptomycin-dependent strain of *S. typhosa*, antigenically similar to the parent strain but low in virulence, capable of stimulating antibody production and capable also of protecting mice against challenge with the parent susceptible strain, no reports have appeared to date to indicate conclusively that isoniazid-resistant strains of tubercle bacilli are avirulent for mice. Indeed, some resistant strains retaining little or no virulence for guinea pigs have been reported to be fully virulent for mice.[7] The data presented herein, with a single strain of

[3] STEENKEN, W., JR., AND WOLINSKY, E.: Am. Rev. Tubere., 1953, *68*, 548.

[4] MIDDLEBROOK, G., AND COHN, M. L.: Science, 1953, *118*, 297.

[5] BLOCH, H., WIDELOCK, D., AND PEIZER, L. R.: Am. Rev. Tubere., 1953, *68*, 734.

[6] REITMAN, M., AND IVERSON, W. P.: Antibiotics Annual, 1954, 604.

[7] MITCHISON, D. A.: Adaptation in Micro-Organisms, Cambridge University Press, England, 1953.

M. tuberculosis, would suggest that emergence of resistance to isoniazid is associated[7] at least in some instances, with a loss of virulence for mice as well as guinea pigs.

The correlation between resistance and degree of virulence has recently been discussed in detail by Mitchison[8] and Stewart[9] who emphasize the significance of mixed populations in this regard. In the present study, the isoniazid-resistant strain utilized was composed of a mixed population with only small numbers of resistant cells. No evidence is available as yet to prove whether or not these resistant cells were responsible for the diminished virulence of the strain. Although genetic association between loss of virulence and development of drug resistance has been established for some bacteria by the recent observations of Hotchkiss,[10] the high proportion of susceptible cells comprising the mutant isoniazid-resistant strain would lead one to suspect that loss of virulence had occurred independently of emergence of isoniazid resistance.

Pierce, Dubos, and their associates[11, 12] have reported partial protection in mice after immunization with attenuated viable strains of *M. tuberculosis* and have shown furthermore that the degree of immunity achieved is dependent upon the extent to which multiplication of the attenuated bacilli takes place *in vivo*. In the present study only partial protection likewise was noted after immunization with the avirulent isoniazid-resistant mutant or with the Phipps BCG strain. The comparable degree of protection achieved with these two strains suggests the possibility that they may multiply at comparable rates *in vivo*, and studies are in progress to evaluate this point.

The possibility that greater protection might have been achieved in the present experiments if the immunizing strain had consisted of a totally resistant population of cells is under investigation. There is no evidence to date, however, that immunity in these animals was produced by the resistant cells in the population. Furthermore, to assume that immunity could be produced in any host merely by contact with such cells would be fallacious, for it is well known that numerous factors operate *in vivo* to enhance or diminish host resistance.

As the data presented herein pertain to effects resulting from administration of mixed populations, and as methods which will detect small numbers of virulent cells are not available, it must be recognized that isoniazid-resistant strains of this nature are not suitable for immunization of humans.

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APRIL 27, 1954

[8] MITCHISON, D. A.: Am. Rev. Tubere., 1954, *69*, 640.

[9] STEWART, S. M.: Am. Rev. Tubere., 1954, *69*, 641.

[10] HOTCHKISS, R.: Bull. of the New York Academy of Medicine, 1952, second series, 28(5), 346-348; Genetic chemistry of the pneumococcal transformers. The Harvey Lectures, published under the auspices of the Harvey Society, New York. Academic Press, Inc., New York, 1954 (in press).

[11] PIERCE, C. H., DUBOS, R. J., AND SCHAEFFER, W. B.: J. Exper. Med., 1953, *97*, 189.

[12] DUBOS, R. J., PIERCE, C. H., AND SCHAEFFER, W. B.: J. Exper. Med., 1953, *97*, 207.

REGRESSION OF TUBERCULOUS LESIONS IN GUINEA PIGS INFECTED WITH ISONIAZID-RESISTANT TUBERCLE BACILLI

In a recent account[1] it was reported that the subcutaneous injection of isoniazid-resistant tubercle bacilli (H37Rv) into guinea pigs resulted in lesions of internal organs which could be seen in animals killed twenty-one days after injection but which could not be seen in those killed eighty-eight days after injection. This was in contrast to the widespread progression of the lesions seen in animals infected with the isoniazid-susceptible strain of H37Rv.

These results were confirmed in a second experiment in which 14 guinea pigs were each infected subcutaneously over the sternum with an isoniazid-resistant culture which had been isolated from the spleen of an animal killed on the twenty-first day in the first experiment. The inoculum was prepared from growth on egg-yolk agar containing 10 γ of isoniazid per ml. Six of the animals were killed on the twenty-first day of infection and were found to have grossly visible lesions of the liver, spleen, site of inoculation, and axillary lymph nodes. On the seventy-second day, the remaining 8 animals were killed and were found to have no grossly visible lesions of the internal organs. There were, however, in each animal an abscess at the site of inoculation and necrosis of axillary lymph nodes, from which isoniazid-resistant tubercle bacilli were isolated.

These observations indicated that the isoniazid-resistant variant of H37Rv which had been obtained *in vitro*[2] was capable of causing in guinea pigs transient lesions which eventually regressed. The same phenomenon has been observed with three isoniazid-resistant cultures isolated from patients who had been treated with the drug.

Culture No. 1: This culture, resistant to more than 10 γ of isoniazid per ml., had previously been considered as avirulent for guinea pigs because no grossly visible lesions were seen eight weeks after the intraperitoneal injection of 0.1 ml. of a 1:50 dilution of culture made in Dubos liquid medium. The inoculum for the experiment described here was prepared from a 21-day-old culture on egg-agar containing 10 γ of isoniazid per ml. Each of 30 mature, male guinea pigs was injected intraperitoneally with 0.1 mg. (moist weight) of the culture. In two weeks, all the animals were sensitive to the intradermal injection of 0.1 ml. of 1:100 Old Tuberculin.

Groups of 6 animals each were killed after two, five, seven, ten, and fourteen weeks, respectively. In the two-week group, there were visible lesions in the liver, spleen, omentum, hepatic, iliac and inguinal lymph nodes, and the epididymis. In five weeks, the lesions were less extensive; 3 animals had no lesions in the liver or hepatic lymph nodes; 2 had normal-appearing spleens. However, the involvement of the mesentery and the lymph nodes and the presence of peritesticular abscesses persisted. At the seventh week, there was no unequivocal evidence of tuberculous lesions except the persistence of peritesticular caseous abscesses. In the groups killed after ten and fourteen weeks, the internal organs were grossly normal but there were abscesses up to 4 mm. in diameter on the epididymis, the testes, and the cremaster muscle of most of the animals. Cultures obtained from these as well as from lesions in animals of each earlier group were resistant to more than 10 γ of isoniazid per ml.

Culture No. 2: This culture was resistant to more than 100 γ of streptomycin and to more than 10 γ of isoniazid per ml. Prior to its use in the following experiment, it was subcultured three successive times on medium containing 10 γ of isoniazid per ml. Each of 30 mature male guinea pigs was infected intraperitoneally with 0.1 mg. of culture

[1] Karlson, A. G., and Ikemi, Y.: Proc. Staff Meet., Mayo Clin., 1954, *29*, 119.

[2] Karlson, A. G., and Ikemi, Y.: Proc. Staff Meet., Mayo Clin., 1952, *27*, 239.

from isoniazid-containing medium. Groups of 6 animals each were killed after two, four, six, eight, and fifteen weeks of infection, respectively. In the two-week animals, grossly visible lesions were present in the spleen and liver, omentum, and peritesticular areas as well as various lymph nodes. In four weeks, it appeared that the disease had progressed; in addition to involvement of other organs, 4 animals had a few pulmonary lesions. In the six-week group, however, only one animal had any evidence of lesions in the spleen and 4 had a few indefinite foci in the liver. There were caseous nodules in the omentum and on the testes and epididymis. In the group killed after eight weeks, all of the spleens appeared normal and 4 animals only had small, indistinct foci in the liver; there were caseous nodules in the omentum and on the testes. One animal had an enlarged hepatic lymph node, and another had a caseous abscess in the abdominal wall at the site of injection. The last group of animals was permitted to live for fifteen weeks. The only visible lesions in the internal organs consisted of a single circumscribed caseous lesion in the liver of one animal and small, firm but caseous nodules in the omentum of 3 and on the testes of 4. Cultures were isolated from lesions of animals in each of the groups, and all were found to be resistant to more than 10 γ of isoniazid per ml. All of the animals were tuberculin-positive when tested two days before being killed.

Culture No. 3: The results with this culture are of interest because it had been passed through guinea pigs and then through mice with an intervening culture on medium containing 10 γ of isoniazid per ml. In the first passage, 10 guinea pigs were given 0.1 mg. subcutaneously over the sternum. In sixty-two days the lesions were limited to the site of injection and the axillary lymph nodes. A culture from an axillary node of one guinea pig was used to infect 8 mice intravenously and 8 guinea pigs intraperitoneally. The mice died but the guinea pigs, killed in twelve weeks, had lesions limited to the omentum and testes. Cultures from the mice and guinea pigs were resistant to more than 10 γ of isoniazid per ml.

A culture from the lungs of one mouse, isolated on medium containing 10 γ of isoniazid per ml., was used to infect 30 mature male guinea pigs, each animal being given 0.1 mg. intraperitoneally. Six animals in the group were killed after two, four, six, eleven, and eighteen weeks, respectively. In animals of the two-week and four-week groups, there were readily visible lesions of the liver, spleen, omentum, testes, and various lymph nodes. After six weeks it was found that 3 of the 6 animals had no gross lesions of the liver or spleen, and one other had 3 small but definite tubercles in the liver. In the group killed after eleven weeks, only one animal had visible lesions in the liver and spleen and another had large caseous nodules in the liver. Involvement of various lymph nodes, omentum, and testes persisted. Because of the presence of lesions even in a few of the animals killed at eleven weeks, the last group was permitted to live for eighteen weeks. Four animals had no lesions of the liver or spleen, one had small nodular lesions in the liver, and another had definite tubercles in liver and spleen. All animals had small fibrocaseous nodules in the omentum and the testes. Although there was evidence that the lesions caused by this culture tended to regress, it appeared to be more virulent than the two described earlier in this paper. Whether or not the previous animal passages had any influence on the virulence of this isoniazid-resistant culture is a matter of speculation.

In this last study, cultures were obtained either from the omentum or the lesions on the testes, and all were found to be resistant to more than 10 γ of isoniazid per ml.

These observations indicate that isoniazid-resistant tubercle bacilli are capable of producing lesions in guinea pigs when given intraperitoneally in a relatively large dose and that such lesions tend to regress.

ALFRED G. KARLSON

MAYO CLINIC
ROCHESTER, MINNESOTA

June 7, 1954

Correspondence

THE POSSIBILITY OF AN ANTAGONISTIC EFFECT BETWEEN PNEUMOTHERAPY AND CHEMOTHERAPY

To the Editor of The American Review of Tuberculosis:

During the year 1953, more than 90 per cent of the patients at the Wm. H. Maybury Sanatorium were placed on a regimen of long-term chemotherapy; no patient was given any type of temporary collapse of the lung during the same period. This means an abrupt reversal of therapeutic policy in an institution which for many years has made use of temporary collapse measures, including surgical interruption of the phrenic nerve.

The complete dependence upon chemotherapy at Maybury is representative of the position taken by many hospitals throughout the United States. Long-term chemotherapy seems to work well. Nevertheless, the individual responsible for therapeutic policy must face the uncomfortable question: might some of the patients receiving chemotherapy alone have profited by the addition of temporary collapse of the lung? This question is made more pertinent in view of the stated opinion of several authorities in the field that in selected cases it should be possible to combine chemotherapy and collapse therapy to the benefit of the patient (Panel discussion: Am. Rev. Tuberc., 1953, *68*, 954). Certainly, pneumothorax in the past has been a mainstay in the control of tuberculosis. Since this is true, why is it not possible to make simultaneous use of pneumotherapy and chemotherapy, each working in a different manner, to the patient's advantage?

The present position of the Maybury Staff in this matter was reached on a pragmatic basis. In the early days of streptomycin therapy, when the development of resistant tubercle bacilli was such a serious problem, a determined effort was made to close all cavities by means of temporary collapse of the lung. During this period the suggestion was made at times that pulmonary collapse might interfere with chemotherapy, but the idea was not generally accepted. In 1951, when long-term therapy with streptomycin and PAS became the standard, each case so treated was reviewed at sixty-day intervals from the point of view of the possible benefit to be obtained if some form of temporary collapse of the lung were added to the chemotherapy. Temporary collapse measures were abandoned rapidly during 1951 and 1952 and were not used at all during 1953, simply because members of the staff experienced in the use of pneumotherapy could not see how any of the individual cases which came before the conference would be benefited by adding temporary collapse of the lung to the chemotherapy.

Aside from practical experience in the clinic and the conference room, recent bacteriologic studies suggest that the acknowledged effectiveness of pulmonary collapse, with sterilization of the lesion in many cases, is probably due to the decreased supply of oxygen reaching the diseased area. It is not necessary that the area should be rendered completely anaerobic, for Dubos has shown *in vitro* that tubercle bacilli under partially anaerobic conditions are highly sensitive to lactic and other acids which are known to accumulate in the body in areas which have a deficient supply of oxygen (Dubos, R. J.: J. Exper. Med., 1950, *92*, 319; 1953, *97*, 357; 1953, *98*, 145). He states: ". . . under conditions of oxygen deficiency, the presence of sodium lactate may result not only in inhibition of growth but also in a bactericidal effect." Thus, it becomes increasingly evident that the sterilization of tuberculous lesions, which is known to have occurred in many cases of successful pneumotherapy and thoracoplasty, has been in large part the result of cutting down the supply of oxygen.

From the point of view of the physician, also, it is generally recognized that any type of pneumotherapy is ineffective unless selective collapse of the diseased area is obtained. As stated by Barnwell: "In a properly managed selective collapse, inspiration and expiration should show no change in volume of the involved area . . ." (*The Collapse Therapy of Pulmonary Tuberculosis*, Charles C Thomas, Springfield, Illinois, 1937). For all practical purposes, this means that the diseased area tends to become and remain atelectatic. Ineffective pulmonary collapse probably causes little or no interference with the circulation of blood about a tuberculous lesion, but the atelectatic condition obtained in selective collapse is a different matter. Under such conditions, the circulation of blood decreases progressively with the lapse of time. This effect of pulmonary atelectasis has been demonstrated recently by Bjork in experiments on human patients (J. Thoracic Surg., 1953, *26*, 533). He showed that acute atelectasis for one hour does not significantly decrease the blood flow through an atelectatic lung but that "After atelectasis for one and one-half months practically no blood was passing through the atelectatic lung."

Evidence, then, from both the bacteriologist and the surgeon points to a marked decrease in the supply of blood and oxygen to a diseased area as the primary result of successful pneumotherapy. This brings to a focus the incompatible nature of pneumotherapy and chemotherapy, for successful use of the latter implies an intact circulation by which the therapeutic agent may be brought to the diseased tissue. The two types of therapy work in different ways, it is true, but they appear to work in ways which are antagonistic. It seems evident that a truly effective type of pneumotherapy, one which produces selective collapse of the lung, tends to inhibit therapeutic concentrations of antimicrobials in the bloodstream from reaching the very area where they are supposed to be effective and that the extent of the inhibition will be proportional to the "successfulness" of the collapse.

The possible incompatibility of chemotherapy and pneumotherapy does not mean that there is no place for the latter in the treatment of tuberculosis. The pendulum has probably swung to the extreme in neglecting temporary collapse measures. For the patient who has become allergic to the best antimicrobials, or who has tubercle bacilli resistant to the drugs, the first thought should be of pulmonary collapse rather than of turning to less effective or more toxic chemotherapeutic agents such as oxytetracycline and viomycin. Having decided to use pulmonary collapse, however, the physician should realize that the pneumotherapy, if successful, may tend to inhibit specific therapy by way of the bloodstream.

Sincerely yours,

Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium)
Northville, Michigan

May 11, 1954

C. Eugene Woodruff
Wilbur J. Steininger
Edna M. Jones
Morris D. Kloffenstein
Edwin E. Mueller
W. Leonard Howard

HAND TALKING CHART

To the Editor of The American Review of Tuberculosis:

In January, 1943, I was stricken by a coronary thrombosis followed by a cerebral embolism, which resulted in a right hemiplegia and complete aphasia. I was a shut-in within a paralyzed body. It was like living behind a glass wall. I had no way to express my needs and necessities. I could hear the physicians and nurses talking about me. But I couldn't let them know I heard or understood.

It was then, by using my left hand, that I devised twenty-one hand signs. It was two and one-half years later, however, before I could express myself orally and have an artist make the drawings that became the "Hand Talking Chart," and for seven years it has been distributed nationally and internationally. The use of the chart has aided those who were left speechless by injuries or by strokes in which they talk with the simple hand signs, as they can move a hand and a few fingers.

To illustrate: Two fingers held upright means "No." Three fingers means "Yes." Four fingers upright, with the thumb laid across the palm, signifies "I understand." The clenched fist, with the knuckles showing, means "I'm hungry." The fist reversed, with the fingers and palm uppermost, means "I'm thirsty." Spread fingers, with the palm down, means "I'm cold." The fingers spread, with the palm up, means "I'm too warm."

The different hand signs carry the information of: Repeat; OK; bedpan or bathroom; I want to go to bed; I want to smoke; I want news, letters from friends; I'm asking for puzzles, games, cards, et cetera; I want to read books, newspapers, et cetera; I want bank book, business papers; I want to sit in chair; I want entertainment, radio, etcetera; I want my glasses; I am asking for doctor, nurse, wife, husband. By this chart, the aphasic paralytic can make himself understood.

It is my purpose that the medical colleagues who have aphasia patients may have the chart if they wish, and there is no charge for a copy of the chart.

Sincerely yours,

601 West 110 Street
New York 25, New York

Hamilton Cameron

May 18, 1954

ANTIMICROBIAL THERAPY AND PROGNOSIS OF PRIMARY TUBERCULOSIS

To the Editor of the American Review of Tuberculosis:

I was very interested to read the paper written by Edith M. Lincoln, entitled, "The Effect of Antimicrobial Therapy on the Prognosis of Primary Tuberculosis in Children", in the May issue of the Review. For some considerable time I have been thinking along the same lines as the author, namely, the advisability of treating asymptomatic primary tuberculosis in children with antimicrobials. To the best of my knowledge, prophylactic treatment is not a generally recognized practice in these cases and I feel that serious consideration should be given to the problem.

According to findings by various workers in this field, streptomycin and PAS therapy is not necessarily effective in preventing the dreaded complication of meningitis or even complications in bones and nodes but, as pointed out by Dr. Lincoln, our thinking in this connection may have to be revised with the advent of isoniazid, a proved effective agent against tuberculous meningitis.

During the past three months, I have had two cases of tuberculous meningitis develop in children, one an infant one month old and the other a child of three and one-half years. The infant was exposed to his mother, who had active cavitary tuberculosis diagnosed shortly after the birth of her baby, and the other contracted his infection from an uncle who lived in the same house and had an active cavitary lesion.

Both of these children had negative roentgenograms and were negative tuberculin reactors at their initial examination: hence, no interference appeared to be indicated but the parents were advised to report any abnormal behavior. It was assumed in both instances that infection had probably taken place, but skin allergy had not yet developed. With this in mind, it was intended to X-ray and skin-test both children within the next

six weeks, but in both instances the children developed meningitis before their recheck period was due. The infant died quickly in spite of rapid and adequate treatment, and the older child is at present under treatment but is in a precarious condition.

I am wondering if it would not be good practice to institute prophylactic isoniazid therapy in situations of this nature and not wait until infection is proved through tuberculin conversion or the appearance of symptoms, which proved so costly in these two cases. It is my personal opinion that, in very young children and infants, when definite contact has been established as in these cases, antimicrobial therapy should be instituted for both positive asymptomatic and negative tuberculin reactors at the earliest possible moment.

A workable formula in the management of these cases is long overdue, and many young lives are lost annually through our inertia. It is hoped that this letter will promote more interest in the problem and stimulate further discussions through your columns.

Sincerely yours,

NEW WESTMINSTER STATIONARY CLINIC

A. HAKSTIAN

CORNER 6TH STREET AND CARNARVON STREETS
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BRITISH COLUMBIA

May 26, 1954

TRAUMATIC INDUCTION OF PNEUMOTHORAX

To the Editor of The American Review of Tuberculosis:

My editorial in the January, 1954, issue of the REVIEW concerning the traumatic induction of pneumothorax became the object of criticism by Dr. W. Weiss in the letter to the editor published in the May issue of the REVIEW.

I would greatly appreciate your publishing my answer to Dr. Weiss's criticism.

In criticizing me, Dr. W. Weiss discusses only a few points of my paper, neglects some of my considerations and statements, and gives a rather incomplete and one-sided picture to those who might not have read my article.

Paradoxically enough, Dr. Weiss's conclusions, in spite of all apparent opposition to my statements, are in some instances very similar, if not identical, with my conclusions. Dr. Weiss objects to my saying that "the majority of investigators consider the accidental pneumothorax as a preventable complication, not as a rule."

In the original form, my manuscript, sent for publication, was not meant for an editorial and, consequently, was much larger and contained rather extensive and unselected bibliography. It is a matter of fact that most of the writers considered traumatic pneumothorax (on clinical and, in some instances, on experimental bases) as a preventable complication and, therefore, I consider Dr. Weiss's assertion that I was improper in stating so completely unjustified.

Stating that "it is difficult to imagine that a needle of any sort does not puncture both the visceral and parietal pleura" and that "there is as yet no good proof to the contrary," Dr. Weiss neglects my description of Markgraf's technique and Markgraf's original article, based on 7,000 initial pneumothoraces and, also, Dr. Reinhard's observation that the lung may respond by an active contraction in the site of the puncture, thus facilitating aspiration of the air from the tube and preventing injury to the lung. Such pulmonary contractions may occasionally be observed during the thoracoscopy, and the evidence of active pulmonary contractivity has support in numerous observations and experiments.

This problem was discussed by me elsewhere (Quart. Bull., Sea View Hosp., 1952, *18*, 162 and 205).

Dr. Tchertkoff and associates, on the material of twenty-nine patients found in *all* cases of initial pneumothorax or even after merely taking pleural readings, a progressively increasing pleural space, or, in other words, they found 100 per cent of accidental pneumothorax proved clinically and roentgenologically. It is self-evident that in all of these cases the gas volume determination would have shown an increase of air space. And, indeed, Ornstein and associates found on five initial pneumothoraces from the same Sea View Hospital an increase of air volume of 410 per cent and 820 per cent in two cases and of 38, 47, and 44 per cent in three others, respectively. There is no doubt that, in these two cases, the X-ray would have shown the enormous increase of air as well as a smaller increase in three others. So one should not wonder that, in the same institution where the same type of patients were used, the results of clinical and physiological experiments have led to the same conclusions. In other institutions (Dooneief and Piccagli, Markgraf, and present writer) using the different technique, different clinical results were obtained and corroborated my belief that the trauma to the lung does not play the first role in the creation of an initial pneumothorax and that, consequently, the lung puncture technique is deprived of a solid foundation.

In his letter, Dr. Weiss stated that: "Of course, in *most* cases, the laceration of the visceral pleura is undoubtedly small and rapidly closes over so that *relatively little air escapes from the lung. Consequently, the small amount of air released from the lung may not be noticed.*" He states further that "It is true that, with a careful technique, traumatic pneumothorax large enough to be of clinical significance does not often occur." Before that, Dr. Weiss stated that he repeated Tchertkoff's experiment, which convinced him in the traumatic origin of initial pneumothorax, or, in other words, he came to the same conclusion as Tchertkoff. Since Tchertkoff states that in *all cases* there was a progressively increasing pneumothorax space which required deflation in a high percentage of cases, one can see an evident contradiction in Dr. Weiss's statements. Moreover, the aforementioned statements by Dr. Weiss (who, incidentally, does not agree with Tchertkoff's pneumothorax induction technique) do not much differ from mine when I stated that: "It can be argued that a certain leakage of air into the pleural cavity can not be excluded in cases where no traumatic pneumothorax could be diagnosed. Even if it were so it should be negligible and not enough to increase the collapse significantly." I never have denied the possibility of a small leakage of air into the pleural space, but merely stated that with a proper and careful technique the clinically and roentgenologically detectable accidental pneumothorax can be prevented and that the direct inflation of air, not trauma, is mainly responsible for the initial pneumothorax space and, consequently, condemned the traumatic induction of pneumothorax.

Therefore, it seems to me that most of Dr. Weiss's criticism, with the accusation of me being one-sided in my expressions, doesn't reach the target, is unjustified, and somewhat superfluous.

Yours very sincerely,

CONSTANTINE VYSNIAUSKAS

MOLLY STARK HOSPITAL
CANTON 1, OHIO

May 11, 1954

The italicized words in some sentences are mine.

Books

ADVANCES IN THE CONTROL OF ZOONOSES. Bovine Tuberculosis, Brucellosis—Leptospirosis, Q Fever—Rabies. WHO/FAO Seminar on Zoonoses, Vienna, November, 1952. Pp. 275, World Health Organization, Geneva, 1953, Price, $3.00.

At times we think of ourselves too much as physicians and as bacteriologists and forget that we are all biologists. Divisions of science are necessary but, none the less, artificial. This report does much to remind us of biology and that human disease may occur as an accident in the natural history of the pathogen which is responsible for disease in animals. In this book are discussed the best methods available for the study, diagnosis, and control of diseases of animals transmissible to man. Five of these diseases thought to be of greatest interest in Europe are discussed. It should be noted that four of them are due to intracellular parasites. The strategy that the control of the disease in animals will lead to prevention or eradication of the disease in man is discussed, and tactical plans to this end are outlined. In all cases this consists of elimination of infected animals or vaccination or both.

In the section on bovine tuberculosis, we find the methods for typing tubercle bacilli and the epidemiologic results of such work in Denmark. Methods of control of the disease and its diagnosis in cattle are presented. Here, as in the human disease, the density of the exposed population affects the incidence of disease so that range cattle are often healthy. Vaccination in cattle with BCG is discussed, only to be dismissed "particularly as, in some cases, deaths had occurred and a degree of stunting had arisen which could be attributed only to vaccination." Descriptions are also included of the examination of milk and of the preparation of Weybridge PPD. Each section is followed by a résumé of the discussions at the meeting. In the discussion on bovine tuberculosis, it is pointed out that bovine tuberculosis in man is more serious than has been generally believed. The respiratory route of infection has often been overlooked. The hemagglutination test for prognosis in cattle, methods to distinguish the saprophyte from the pathogen, and false tuberculin reactors were all discussed.

Throughout all sections of the book, methods and techniques are described with a craftsmanlike skill. Thus, the manual is a compendium of great value to many workers. Nonetheless, one is left with a feeling of the great need for simpler and quicker methods. One point of criticism is the delay between the meeting and the publishing of this report.

The rest of the book is full of matter of great interest to the student of infectious disease. A progress report on the gradual elimination of brucellosis in cattle is included. Interesting problems as yet unsolved are mentioned, such as bursts of infectivity in Q fever-carrying animals. Other diseases in animals thought to be caused by the leptospira strains are discussed.

This is a book of great interest to many workers in diverse fields and it has succeeded in uniting medical and veterinary information for the benefit of practitioners of both arts.

J. MACLEAN SMITH
New York, New York

LES ANIMAUX DE LABORATOIRE (Anatomie, Particularités physiologiques, Hématologie, Maladies naturelles, Expérimentation). By Julien Dumas. Pp. 720, Flammarion, Paris, 1953, Price, $12.00.

This remarkable book should prove indispensable to all investigators—and especially so to the microbiologists—whose work involves animal experimentation. We do not know of a more comprehensive treatment of the subject in any language, yet it is presented very concisely, with constant attention to practical problems.

The first section describes the anatomy, physiological peculiarities, hematology, and breeding conditions of various laboratory animals (guinea pig, rabbit, mouse, rat, hamster, ferret, spermophile, hen, pigeon, monkeys, cat, and dog).

The second section contains 50 pages of valuable information on the housing and feeding of these animals, as well as on types of cages and their cleaning and disinfection.

The third section describes, in great detail, but in a concise and very orderly way, the various bacterial, viral, protozoan, and helminthic diseases naturally occurring in the guinea pig, rabbit, mouse, rat, ferret, hen, and the monkeys. The consequences of avitaminosis, the tumors, and the ectoparasites are also described.

For each disease, the author discusses the symptoms, the anatomo-pathologic and histologic findings, the diagnosis (with emphasis on the specific identification of the causative agent), the experimental reproduction of the disease, its epidemiology and prophylaxis.

The fourth section describes the techniques of animal experimentation: marking, handling, anesthesia, collection of samples, modes of inoculation, autopsy, and sanitary disposal of the corpses.

The book contains 163 drawings, sketches, and photographs, and a good alphabetical index. After each chapter, there is a complete list of references, truly international in scope. The printing and the paper are excellent.

G. H. WERNER
Brooklyn, New York

BOOKS RECEIVED

MANUAL OF TROPICAL MEDICINE. Second Edition. By Thomas T. Mackie, George W. Hunter, and C. Brooke Worth. Pp. 907 with illustrations, W. B. Saunders Company, Philadelphia and London, 1954, Price, $12.00.

ANNUAL REVIEW OF MEDICINE. Volume 5. Pp. 490, Annual Reviews, Inc., Stanford, California, 1954, Price, $7.00.

GREATER WORKS. Stories and Articles on the Cure of the Sick and the Care of the Unfortunate. Compiled and arranged by Leona Meyer Wegener. Pp. 246, Exposition Press, New York, 1954, Price $3.50.

American Trudeau Society

Medical Section of the National Tuberculosis Association

Report of Committee on Therapy to the Committee on Medical Research, May, 1954

The American Trudeau Society Committee on Therapy met three times during the year 1953–1954. The first meeting was held in New York on October 14–15, 1953, the second meeting in St. Louis on February 6, 1954, and the third meeting in Atlantic City on May 13–14, 1954. Two joint meetings with the Laboratory Subcommittee were held; the first in New York in October and the second in Atlantic City in May. All members attended one or more meetings.

Because the composition of the committee was largely new, the major portion of the first meeting was devoted to planning for the coming year. At the second and third meetings various members reported on the subject assigned them, and a number of statements were prepared for publication in THE AMERICAN REVIEW OF TUBERCULOSIS.

Preparation of a Manual on Pembine-Type Conference

The success of the Pembine Conference has attracted much attention and conferences of the Pembine type have been inaugurated in many parts of the country. Some groups have been interested in starting such conferences, but have been uncertain about the methods and procedures which should be used. To help such groups, the Committee on Therapy undertook the preparation of a guide for the planning of a Pembine-Type Conference. Dr. Morris Thomas presented a preliminary draft of a manual at the October meeting, and a final draft was accepted at the February, 1954, meeting.

Following the winter meeting, it was agreed that the publicity for the manual and distribution would be handled by Dr. Julius L. Wilson, the Director of the Division of Medical Education. It was also agreed that the manual would be shortened and the title would be changed to "Manual for Consecutive Case Conference—(Pembine type)."

Bed Rest in the Treatment of Tuberculosis

The widespread use of potent antimicrobial agents has resulted in considerable confusion concerning the role of bed rest in the treatment of tuberculosis. A statement on the subject was prepared by Dr. Robert Yeager and accepted at the February meeting of the Committee, with the recommendation that it be published in THE AMERICAN REVIEW OF TUBERCULOSIS. It was emphasized that the value of bed rest was known, that the efficacy of ambulatory chemotherapy had not been established, and that, until such time as further data is available, patients with active tuberculosis should receive the benefit of bed rest. The statement will be published in the June issues of THE AMERICAN REVIEW OF TUBERCULOSIS and the NTA *Bulletin*. (This was done. -*Ed.*)

Tuberculous Meningitis

Tuberculous meningitis remains a formidable disease in spite of advance in therapy. As a continuation of the work of last year's committee, it was decided to prepare a statement offering a guide to the physician treating the occasional case of meningitis. Dr. Paul Chapman presented a preliminary draft of the statement at the February meeting, and a final draft was accepted by the Committee in May, with the recommendation that

it be published in THE AMERICAN REVIEW OF TUBERCULOSIS. The statement emphasizes the importance of early diagnosis and prompt treatment. The principles of chemotherapy are discussed, with specific recommendation concerning the drugs to be used and duration of therapy. Methods of relieving block are reviewed.

Tuberculosis of the Eye

Dr. Roger Mitchell reviewed the available literature on therapy of tuberculosis of the eye and sought opinion of some ophthalmologists interested in the subject. He discovered that in the country short-term chemotherapy is being used. It was the consensus of the committee that a seminar on tuberculosis of the eye be prepared for the 1955 annual meeting of the ATS and that ophthalmologists interested in this subject be asked to participate.

Tuberculous Lymphadenitis

Tuberculous lymphadenitis is seen often enough to present a problem in therapy; yet, there seems too little agreement on the best methods of treatment. Dr. Carl Muschenheim was asked to review literature on the subject and present a report to the Committee on Therapy. This was received and approved at the May meeting in Atlantic City, and it was recommended that it be published in THE AMERICAN REVIEW OF TUBERCULOSIS. The report concluded that tuberculous lymphadenitis should be considered a manifestation of a generalized disease and not simply as a local disease process, and that antimicrobial therapy is indicated in most instances of active tuberculous lymphadenitis.

Surgery of Tuberculosis

At the February meeting of the Committee on Therapy, Dr. Raymond Corpe presented a comprehensive statement on the problems of evaluating both collapse and resectional surgery today. Dr. Corpe prepared a formal report on this subject for the May meeting, and the report was discussed at some length. It was finally decided that a formal statement by the committee should not be issued at this time, since any such statement would be rather diffuse and of little practical value.

Tuberculosis in Children

At the October meeting, correspondence from Dr. Dwain Walcher of the University of Indiana was read concerning the lack of interest of pediatric societies in tuberculosis. Dr. Walcher asked in his letter for "comments on the advisability of attempting to initiate a movement through one of the pediatric organizations, thereby integrating and coordinating some of our efforts and thought on the entire problem of tuberculosis in children."

Dr. Walcher met with the Committee on Therapy the entire morning of its meeting on May 14. He discussed at some length the wide differences in opinion among pediatricians concerning the therapy of tuberculosis in children. He again stressed the importance of interesting pediatricians in the general problem of tuberculosis. After general discussion, the committee made the following recommendations:

a. that a pediatrician interested in tuberculosis be asked to serve on the Committee on Therapy,

b. that efforts be made to have a panel discussion on the treatment of tuberculosis at the next meeting of the American Academy of Pediatrics,

c. that efforts be made to have editorials written in THE AMERICAN REVIEW OF TUBERCULOSIS, the *Journal of Pediatrics*, and the *Journal of the American Medical Association* on the problems of treating childhood tuberculosis.

Dr. Julius L. Wilson attended a portion of the May meeting and agreed to help implement the cooperation between the American Trudeau Society and the American Academy of Pediatrics.

It was suggested that next year's committee prepare a statement on the treatment of tuberculosis in children.

Dr. Muschenheim suggested that statements made by the committee be signed only by the chairman instead of the members as has been customary in the past. The committee agreed to this principle.

At the joint meetings with the Laboratory Subcommittee, the Committee on Therapy was informed of recent developments in the laboratory. The problem of avirulence of isoniazid-resistant bacilli was discussed at length.

PAUL T. CHAPMAN
RAYMOND F. CORPE
ROBERT A. GOODWIN, JR.
ROGER S. MITCHELL
CARL MUSCHENHEIM
EDWARD F. PARKER
MORRIS C. THOMAS
ROBERT L. YEAGER

ROBERT H. EBERT, *Chairman*
Committee on Therapy

Obituaries

CARL MULKY

1877–1954

Dr. Carl Mulky, of Albuquerque, New Mexico, died on January 14, 1954, at the age of seventy-seven. He was graduated from Rush Medical College in 1901; licensed in 1919. During World War I he was Captain, U. S. Army Medical Corps, serving as tuberculosis consultant. He was a Fellow of the American Medical Association, of the American College of Physicians, of the American College of Chest Physicians (Governor for New Mexico); and a member of the American Trudeau Society. He was chief consultant, New Mexico State Tuberculosis Sanatorium; consultant, U. S. Indian Service (United Pueblos Area); consultant, Veterans Administration Hospital, Albuquerque; a member, active staff, of Presbyterian Hospital, Albuquerque; associate staff member of St. Joseph Hospital, Albuquerque; president of the New Mexico Trudeau Society; past president of the Bernalillo County Medical Society; past president of the New Mexico State Medical Society; past president of the New Mexico Tuberculosis Association. He had been since 1920 in private practice in Albuquerque as a specialist in chest diseases.

E. B.

WILLIAM HENRY THEARLE

1884–1953

Dr. William Henry Thearle, of Albuquerque, New Mexico, died on December 3, 1953, at the age of sixty-nine. He was graduated from the College of Physicians and Surgeons, Baltimore, Maryland, in 1908; licensed in 1933. He was a Diplomate of the American Board of Surgery; a member of the American College of Surgeons, American College of Chest Surgeons, American Association for Thoracic Surgery, American Trudeau Society, California Medical Association, New Mexico Medical Society, and American Medical Association.

E. B.

HENRY A. JEGI

1873–1953

Dr. Henry A. Jegi, of Galesville, Wisconsin, died on June 27, 1953, at the age of eighty. He was graduated in medicine in 1896 from the University of Illinois, College of Medicine, Chicago. He was a Fellow of the American Medical Association and a member of the American Trudeau Society. He organized the first Wisconsin Seal Sale and was deeply interested in tuberculosis control.

E. B.

IRA F. THOMPSON

1877–1953

Dr. Ira F. Thompson, of Beloit, Wisconsin, died on July 5, 1953, at the age of seventy-five. He received his medical degree in 1900 from Bennett Medical College, Chicago, Illinois. He was a Fellow of the American Medical Association, a member of the American Public Health Association, the American Trudeau Society, and the 50-Year Club of Wisconsin State Medical Society. From 1937 to 1950, he was director of the Wisconsin

Anti-Tuberculosis Association and was its president from 1945 to 1948. At the time of his death, he had been retired from the active practice of medicine for several years.

E. B.

MICHAEL R. WILKINSON

1864–1953

Dr. Michael R. Wilkinson, of Oconomowoc, Wisconsin, died on March 15, 1953, at the age of eighty-eight. He received his medical degree in 1893 from Northwestern University Medical School, Chicago, Illinois. He was an associate member of the American Medical Association and a member of the American Trudeau Society. For nearly sixty years, he was a medical and civic leader in Wisconsin. One of his many interests was diseases of the chest, particularly tuberculosis.

E. B.

SIEGFRIED M. SCHOENFELD

1896–1953

Dr. Siegfried M. Schoenfeld, of New Hyde Park, New York, died on November 16, 1953, at the age of fifty-seven. He received his medical degree in 1921 from the Medizinische Fakultat of the University of Vienna, Austria. He was a member of the American Medical Association, the American College of Chest Physicians, and the American Trudeau Society. At the time of his death, he was on the staff of the Terrace Headquarters Hospital in Hollis, New York.

A. S. P.

ALEXANDRE BRUNO

1889–1953

Dr. Alexandre Bruno, of New York, New York, died in 1953, at the age of sixty-four. He received his medical degree from the Columbia University College of Physicians and Surgeons in 1913. He was a Fellow of the American College of Surgeons, a member of the American Medical Association and the American Trudeau Society. During World War I he was a surgeon with the American Red Cross and received the Cross of the Legion of Honor from the French Government. At the time of his death, he was in private practice.

A. S. P.

SUTHERLAND ERIC R. SIMPSON

1898–1954

Dr. Sutherland Eric R. Simpson, of Watertown, New York, died on January 8, 1954, at the age of fifty-five. He received his medical degree in 1925 from the Harvard Medical School. He was certified by the National Board of Medical Examiners. He was a Fellow of the American College of Physicians and the American Medical Association. At the time of his death, he was medical superintendent of the Jefferson County Sanatorium and a member of the staff of the House of Good Samaritan and Mercy Hospital.

A. S. P.

NOTES

INTERIM SCIENTIFIC SESSION OF THE AMERICAN TRUDEAU SOCIETY

The American Trudeau Society will hold an Interim Scientific Session at the Hotel New Yorker, New York City, on November 17, 1954, from 9 a. m. to 5 p. m. The meeting will be open to physicians interested in pulmonary diseases in internal medicine, surgery, and pediatrics, and to scientists in allied disciplines.

The program will consist of a series of presentations on various aspects of pulmonary diseases from the clinical laboratory standpoints. A detailed program will be announced at a later date.

Persons interested in participating in the program may submit abstracts of material to be presented for selection for the program. Such abstracts should not exceed 200 words in length, and should be typewritten double-spaced and submitted in quadruplicate. The deadline for submission is October 1. The abstracts should be addressed to:

Dr. William B. Tucker, Chairman
Interim Scientific Session Program Committee
Veterans Administration Hospital
Durham, North Carolina.

It is anticipated that notification of selection for participation will be sent to authors submitting abstracts by October 15.

Titles may be submitted on any subject in pulmonary disease, from either a laboratory or clinical standpoint. Not more than twelve minutes will be allotted for presentation. Adequate time for discussion of each paper has been planned. Manuscripts need not be submitted for publication. Investigative work in progress may be reported, but papers should represent original investigative work. Inquiries about further details will be welcome.

COURSE IN LABORATORY DIAGNOSIS OF TUBERCULOSIS

In cooperation with the Division of Special Health Services, Public Health Service, the Bacteriology Laboratories of the Communicable Disease Center, Chamblee, Georgia, will offer a course in the laboratory diagnosis of tuberculosis on the following dates:

November 15–26, 1954

This course is open to all grades of employed laboratory personnel who are approved by their state health officers. Practical laboratory training in all phases of tuberculosis bacteriology, including preparation of culture media, microscopy, cultural procedures, diagnostic use of animals, and testing of drug susceptibility will be included in the course.

Laboratory directors and senior laboratory staff members may also make application for this course.

No tuition or laboratory fees are charged. Reservations for this course should be made well in advance.

Application forms may be obtained from Laboratory Training Services, Communicable Disease Center, Public Health Service, P. O. Box 185, Chamblee, Georgia.

MEDICAL RESEARCH FELLOWSHIPS

The Division of Medical Sciences of the National Academy of Sciences-National Research Council is accepting applications for postdoctoral research Fellowships for 1955–

1956. These awards are designed to offer research experience for promising individuals who look forward to investigative careers, and not to provide practical experience in the clinical field. Ordinarily Fellowships are not granted to persons more than thirty-five years of age. The following programs are announced:

Fellowships in Cancer Research are awarded by the American Cancer Society on recommendation of the Committee on Growth of the Division of Medical Sciences. Awards are available for study in all branches of the biological, chemical, and physical sciences and of clinical investigation applicable to the study of growth, typical or malignant. Citizens of the United States are eligible.

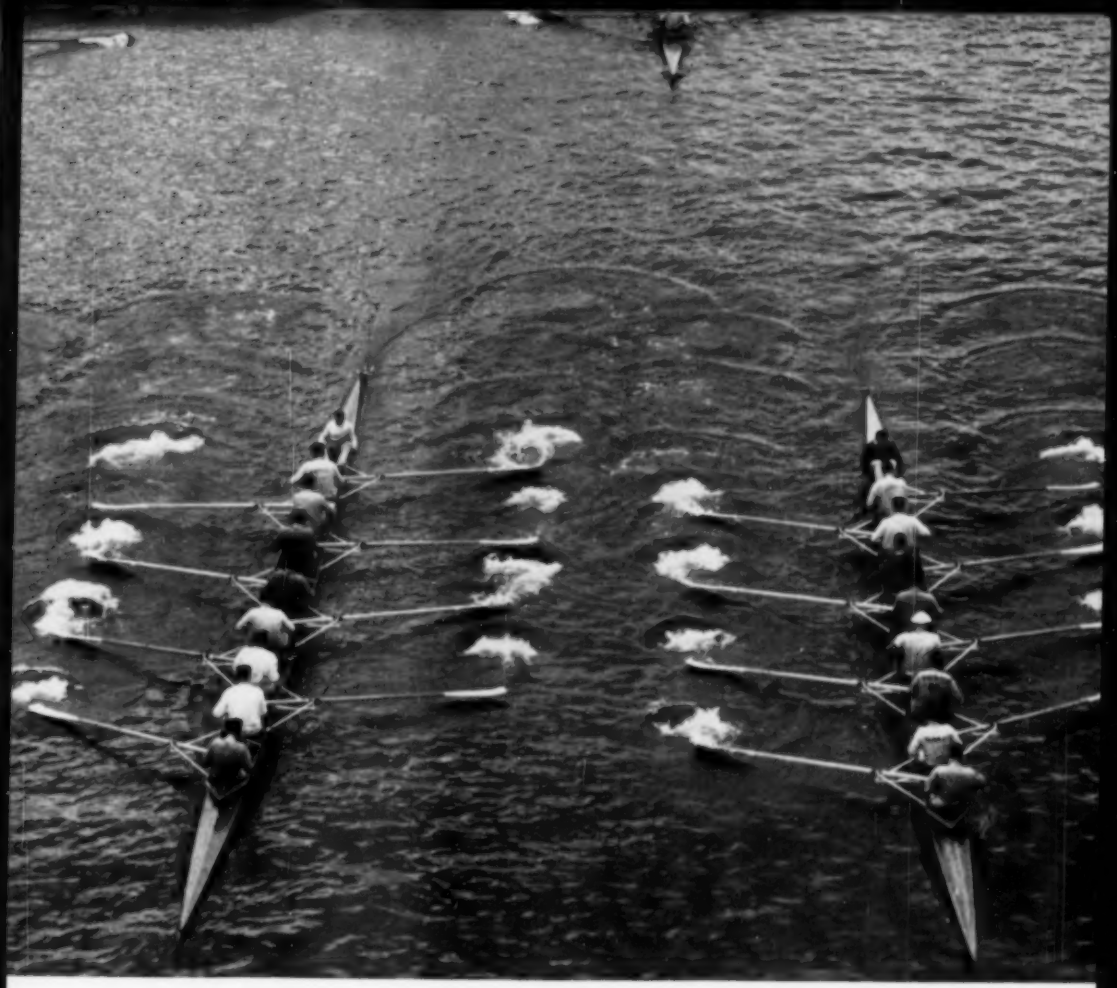
British-American Exchange Fellowships in Cancer Research also are awarded by the American Cancer Society upon recommendation by the Committee on Growth. They are offered to citizens of the United States for advanced study in Great Britain in specialized fields pertaining to the problem of growth. Similar Fellowships are awarded by the British Empire Cancer Campaign to young British scientists for research in the United States.

Fellowships in the Medical Sciences, supported by The Rockefeller Foundation and by The Lilly Research Laboratories, are administered by the Medical Fellowship Board of the Division. Fellows are expected to devote themselves to research in the basic medical sciences. The Fellowships administered for The Rockefeller Foundation are open to citizens of the United States and Canada; the Lilly Fellowships, only to citizens of the United States.

Fellowships in Tuberculosis are also administered by the Medical Fellowship Board under a grant from the National Tuberculosis Association. These awards are designed to promote the development of investigators in fields related to tuberculosis. They are open to citizens of the United States who are graduates of American schools.

Fellowships in Radiological Research are administered for the James Picker Foundation by the Division's Committee on Radiology. The Foundation has expressed particular interest in the support of candidates who propose to carry on research oriented toward the diagnostic aspects of radiology. Appointments are not limited to citizens of the United States.

Applications for 1955–1956 under any of these programs must be postmarked on or before December 10, 1954. Fellowships are awarded in the early spring. Complete details and application blanks may be obtained from the Fellowship Office, National Academy of Sciences-National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D. C.



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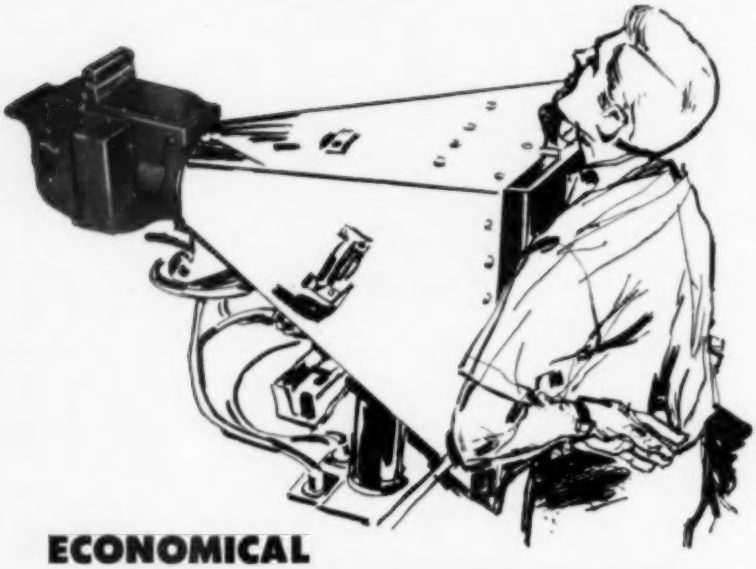

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Report of the Council of Tuberculosis Committees, American College of Chest Physicians. April, 1951:

". . . We also urge routine chest x rays of all admissions to general hospitals. The value of this has been demonstrated in several large hospitals throughout the country where unrecognized cases of tuberculosis and other chest conditions are being discovered."

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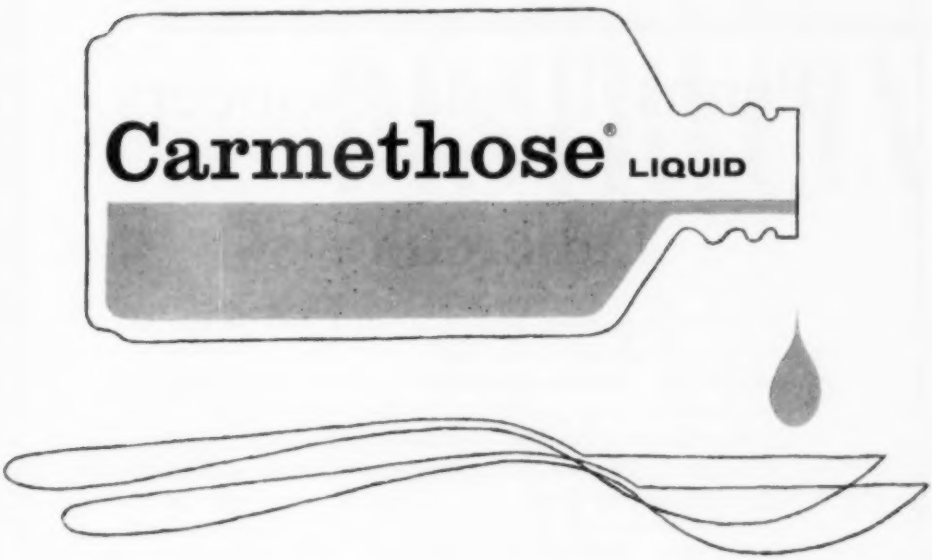

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By Esther Gaskins Price

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When this crusade started, tuberculosis was claiming human lives at the rate of more than 20,000 in a single decade in Philadelphia alone. Popular superstitions, medical conservatism, the hostility of landlords, industrialists and politicians, were some, but by no means all, of the difficult road blocks which confronted the pioneers.

Original Sources

Utilizing the proceedings of some of the most important medical meetings of the 19th century—the archives of the Pennsylvania Tuberculosis and Health Society and public health departments, newspaper files, and family records—PENNSYLVANIA PIONEERS AGAINST TUBERCULOSIS gives the historic details of the long drawn out battle waged by pioneers in the tuberculosis control movement.

This battle, spearheaded by Lawrence Flick and a small but forward-thinking group of earnest co-workers, finally led, among many other specific results, to the creation of the original tuberculosis society in the United States and the establishment of the first institution in medical history to concentrate on the prevention and treatment of a single illness.

Readers of the American Review of Tuberculosis will find in this book interesting and valuable background data, never before available, covering the critical period in which effective health control measures first began to assume their present shape.

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Dihydrostreptomycin	6	0	6
Distrycin	0	0	0
	Cochlear damage % of patients		
	Mild	*Moderate*	*Total*
Streptomycin	0	0	0
Dihydrostreptomycin	12	3	15
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*Heck, W.E.; Lynch, W.J., and Graves, H.L.: Acta oto-laryng. 41:416, 1953.

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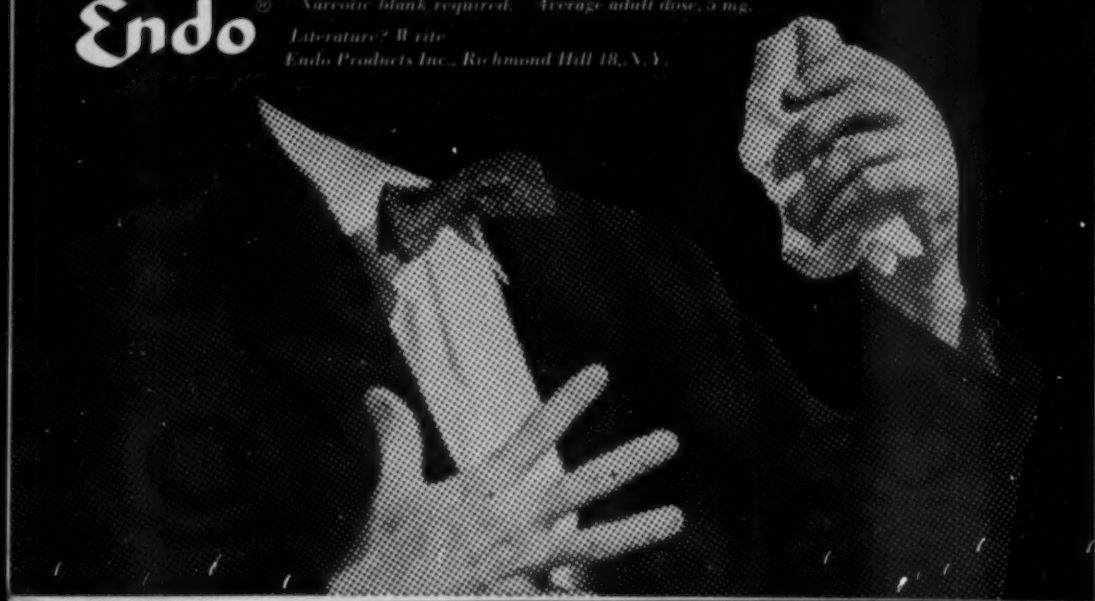
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